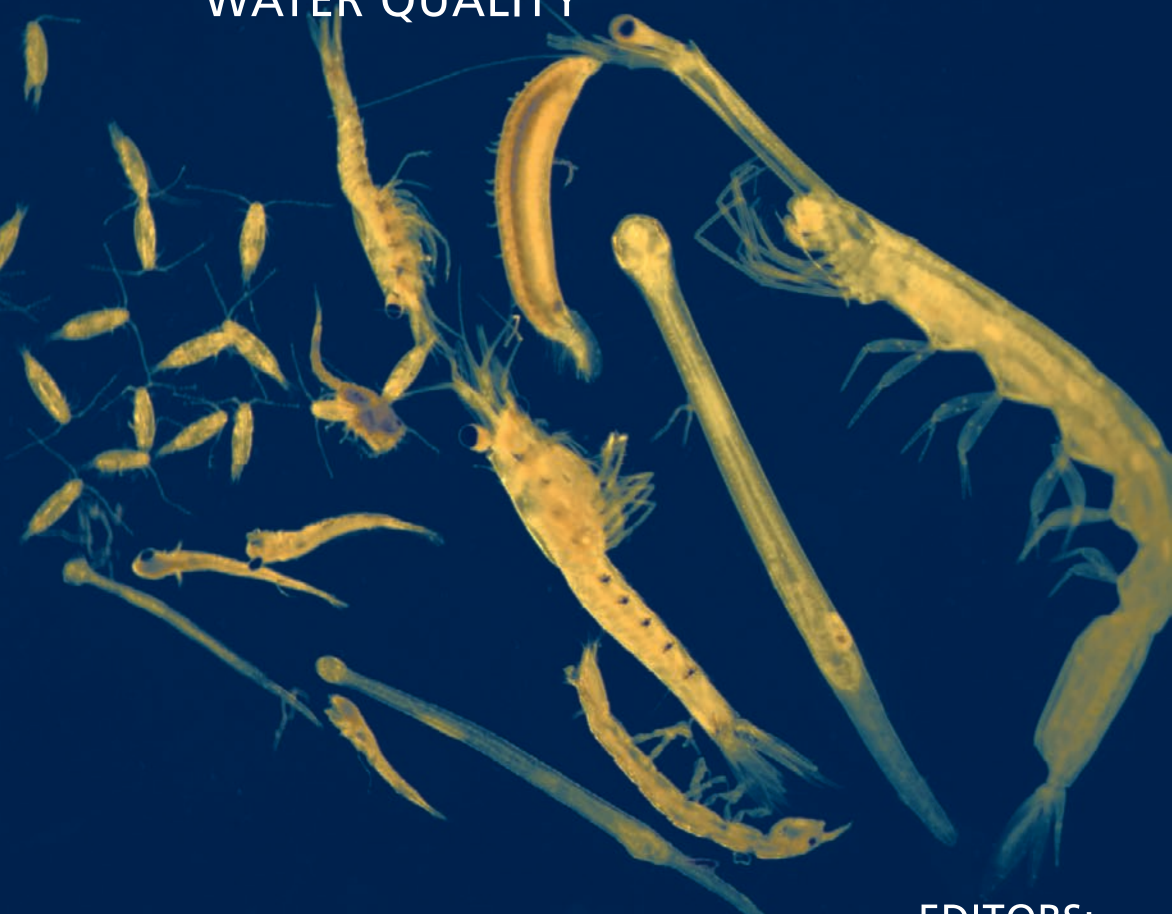
PLANKTON

A GUIDE TO THEIR ECOLOGY AND MONITORING FOR WATER QUALITY

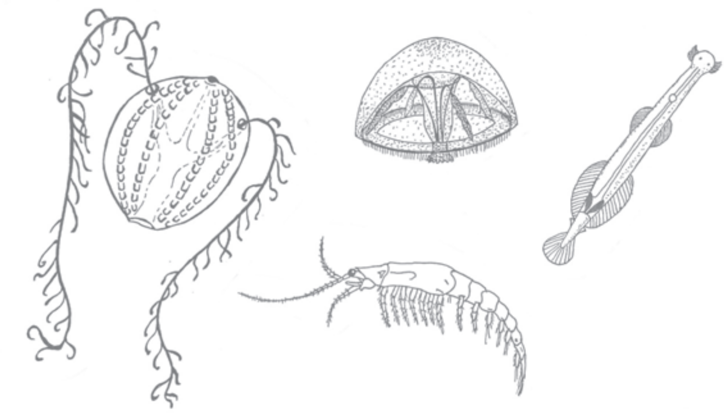


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A guide to their ecology and monitoring for water quality



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CSIRO
PUBLISHING

National Library of Australia Cataloguing-in-Publication entry
Plankton: a guide to their ecology and monitoring for
water quality / editors, Iain M. Suthers, David Rissik.
Collingwood, Vic. : CSIRO Publishing, 2008.
9780643090583 (pbk.)
Includes index.
Bibliography.
Plankton – Ecology.
Water quality management.
Suthers, Iain M.
Rissik, David.
CSIRO Publishing.

577.76

Published by
CSIRO PUBLISHING
150 Oxford Street (PO Box 1139)
Collingwood VIC 3066
Australia

Telephone: +61 3 9662 7666
Local call: 1300 788 000 (Australia only)
Fax: +61 3 9662 7555
Email: publishing.sales@csiro.au
Web site: www.publish.csiro.au

Front cover image by Iain Suthers
All illustrations are by the authors unless otherwise specified.

Set in 10.5/13 Times New Roman
Edited by Peter Storer Editorial Services
Cover and text design by James Kelly
Typeset by Planman Technologies India Pvt. Ltd.
Printed in Australia by Ligare

CSIRO PUBLISHING publishes and distributes scientific, technical and health science books, magazines and journals from Australia to a worldwide audience and conducts these activities autonomously from the research activities of the Commonwealth Scientific and Industrial Research Organisation (CSIRO).

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PREFACE

Many local councils and estuary managers collect phytoplankton and zooplankton in response to the increasing incidence of algal (phytoplankton) blooms in estuaries and coastal waters. In addition, recent studies have shown that the biomass of algae is a better indicator of nutrient stress in waterways than nutrient concentrations. Unfortunately, there has been a lack of consistency and scientific rigour in the methodologies used for sampling, which has often resulted in unresolved outcomes. Monitoring studies are often poorly designed and are ad hoc – making it difficult to identify an appropriate management response. We wish to provide a guide for those preparing or maintaining a water-quality program, as well as to educate people about plankton. By increasing the general awareness about the inhabitants of our water, we can tackle many water quality issues.

The objectives of this guide are:

- to introduce plankton as indices of water quality to managers and students
- to assist in the identification and understanding of plankton from a non-specialist perspective
- to enable people to design, implement and conduct a meaningful plankton sampling program, which may accommodate future changes in technology and respond to new concepts, needs and ideas over time.

This guide is intended for those concerned with water quality and resource management in state government, local governments (council engineers, town planners and landscape architects), community groups (Landcare, Rivercare), environmental consultants and teachers. Management concerns and case studies are key features of this guide to demonstrate the utility of plankton studies for water quality management. We use organism size to introduce the bewildering complexity of plankton, but limit our description to those organisms that can be resolved with a typical microscope or even hand lens. Our target readership includes those without large budgets, and who probably operate from 3–6 m boats, and who may have limited experience with marine or freshwater sampling programs. This guide is written for the curious non-specialist, and contains a moderate reference list.

ACKNOWLEDGEMENTS

Many insights to potential problems in plankton sampling have come from undergraduate teaching during field trips to Jervis Bay, Smiths Lake and Botany Bay, as well as our research along the New South Wales coast. We are very grateful to our colleagues at UNSW – especially to Pat Dixon and Jason Middleton – and our colleagues at the EPA and DLWC (now the NSW Department of Environment and Climate Change and the NSW Department of Water and Energy). Much of our research and experiences for this book were funded by various grants from the Australian Research Council. Mike Kingsford aided our transition from an oceanographic vessel, to quantitative sampling from an open boat.

Anthony Richardson, Glenn McGregor and Brian Griffith gave considerable comments on the penultimate draft (but any remaining mistakes are all our own). The authors of chapter 7 would like to thank I.A.E Bayly, B.C. Chessman, W. Foissner, J.J. Gilbert, D.J. Patterson and B.V. Timms for comments on the early manuscript, B. Atkins for editorial help and I. Faulkner for the illustrations of copepods (Figure 7.2). The authors of chapter 9 would like to thank the Bathurst City Council, and T. Cox of the Ben Chifley Catchment Steering Committee, for help in the project.

We thank the sponsors of this publication including the Queensland EPA, NSW DECC and DWE.

Iain and David dedicate this book to their patient wives, Karen and Chantelle.

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Chapter 1
The importance of plankton

David Rissik and Iain Suthers

Phytoplankton and zooplankton – tiny drifting plants and animals – are vital components of the marine and freshwater aquatic food chains, and our waterways. Plankton communities reflect the effects of water quality and cannot isolate themselves as oysters do by closing their shells in adverse conditions. Plankton are effectively our aquatic 'canaries-in-a-cage' – they accumulate over days the effects of hourly changes in water quality.

In order to manage water quality, we need a broad understanding about plankton and their interaction with the environment. Phytoplankton respond within days to changes in light or nutrients and sediment load, and in response to grazing by larger zooplankton. Therefore from a manager's perspective, the response time of plankton occurs at a meaningful scale compared with changes in water quality, which occur at very small scales of minutes or metres, or changes in the benthic community, which occur at very large scales (weeks or kilometres). The amount of phytoplankton in the water can inform managers about the health of their waterways and where a management action may be required. The types of plankton present in the water are important. For example, only a small number of phytoplankton species are toxic and can be harmful to higher order consumers, such as humans, but not necessarily to the vectors of the toxin, such as oysters or fish. It is important to know something about these species to be able to manage causes of blooms.

1.1 WHAT ARE PLANKTON – AND WHY STUDY THEM?

The term plankton refers to any small biota (from microns to centimetres) living in the water and drifting at the mercy of currents – ranging from bacteria to jellyfish. This definition is rather loose, as we often include jellyfish and krill (euphausids – and their larval forms) as plankton, yet they are active swimmers and are therefore technically referred to as 'nekton'. Sometimes even good swimmers, such as late-stage fish larvae are incorrectly termed 'planktonic', as they often show up in the plankton net, particularly at night. Another definition of plankton is simply 'that material which is caught in a fine mesh net'!

Phytoplankton, such as diatoms and dinoflagellates, grow in the presence of sunlight and nutrients such as nitrogen and phosphorous. These single celled organisms are the 'grasses of the sea' and form the basis of ocean productivity. Some of these plants – but not all – are in turn grazed by zooplankton, which is dominated by small crustaceans such as copepods, shrimps and their larvae. The amount of phytoplankton in the water column reflects the influence of a number of environmental factors and processes. These competing processes may be summed up as 'bottom-up', such as those caused by nutrients and light, or 'top-down', such as those caused by copepods or other grazers.

The majority of chlorophyll in Australian coastal waters is found in the very smallest of cells – the size of bacteria. Their high surface-area-to-volume ratio allows them to out-compete larger cells in the race for nutrients. Most phytoplankton contain photosynthetically active pigments, such as chlorophyll, which enable them to use energy from sunlight to convert carbon dioxide into complex organic molecules, such as sugar or protein (that is, they are autotrophs). Exceptions abound where some of these 'plants' do not fix their own carbon, but engulf and consume other plant cells (that is, they are heterotrophic). Other phytoplankton may be considered as villains – producing red tides or toxic algae – but there are only a few species responsible. Most phytoplankton are enormously beneficial, such as those used in the aquaculture industry.

In the presence of surplus nutrients, zooplankton grazers may be overwhelmed by rapid exponential growth of some phytoplankton ('bloom') over and above what the ecosystem can assimilate. Nutrients that encourage blooms are discharged from river run-off, sewage discharge, stormwater run-off and from groundwater. The surplus of nutrients in waterways, together with the resultant increase in biomass and altered ecology, is referred to as 'eutrophication'. Eutrophication was described in one report as possibly the greatest single threat facing the coastal environment in Australia.

It is important to remember that many phytoplankton blooms may occur naturally: they may be stimulated during the spring, or by natural events such as rainfall or upwelling. Usually, phytoplankton and zooplankton bloom during the early and late summer period, prompting public concern. And yet springtime blooms of the blue-green phytoplankton and gelatinous salps – as well as red tides of a particular dinoflagellate off eastern Australia – are all examples of natural events (see Box 1.1).

Nutrient assimilation by plankton, and nutrient accountability (is the event natural or induced by humans?), underscore the need for using

BOX 1.1 RED TIDES FORMED BY *NOCTILUCA*

The major contributor to red tides off the Sydney coast is an unusual single-celled alga – *Noctiluca scintillans*. Although classified as a dinoflagellate, it has no photosynthetic pigments and feeds at night on other phytoplankton, small zooplankton and their eggs. It contains no toxins, other than a dilute solution of ammonium chloride, which, in large quantities, can irritate the skin and cause localised fish kills. During the final senescent stages of its life, the alga swells up to a comparatively large size of 2 mm diameter and becomes buoyant, thus concentrating at the surface as a reddish, or even bright pink, stain. Its presence year-round off Sydney was never observed before the 1990s, and the frequency of red tides intensified when Sydney's three deep-ocean sewage outfalls were commissioned. Estuaries around the world frequently report *Noctiluca* blooms in eutrophic waters. Was coastal eutrophication stimulating the growth of phytoplankton prey for *Noctiluca*, thus increasing the frequency of blooms?

A major clue was the diameter of cells – small cells indicate cell division and increasing abundance, whereas large cells indicate senescence and are prone to advection by wind, transporting them far from the bloom's cause. The incidence of prey inside the cells also highlighted the importance of the East Australian Current and favourable winds, which transported the cells from areas prone to nutrient upwelling well north of Sydney. In only one case was there clear evidence of small well-fed cells near a coastal sewage outfall. So, what had caused the recent year-round abundance and increased reports of red tides? One reason is the more environmentally aware public during the 1990s, which was keen to report any unusual observations. Also, El Niño events and warming of coastal waters, particularly in 1997–1999 enabled *Noctiluca*'s optimal temperature of ~20°C to be achieved off Sydney.

plankton in a study of water quality. In short, we need to examine and monitor the plankton because:

- some phytoplankton produce toxins that become concentrated in filter-feeding animals such as oysters, mussels and even fish
- phytoplankton may assimilate surplus nutrients, which may be grazed by zooplankton and productively pass them up the food chain to fish
- the early life stages of mussels, oysters, prawns and fish all live in the plankton
- some species of phytoplankton or zooplankton can be indicator species of environmental health by, in effect, integrating the conditions of the past few days or weeks
- the chemical attributes of plankton (such as lipids or natural isotopes), and even their shape or health, can indicate if the eutrophication is natural or human-induced.

These issues will be addressed in the following pages. Water quality is of great concern to the managers of estuaries and coastal waters because unsavoury swimming conditions, poor fishing and bad press translate into reduced spending by tourists and reduced community pride.

Natural Resource Management is a rapidly expanding field, which is increasingly underpinned by better science. In Australia, studies such as the Port Philip Bay Study (Harris *et al*. 1996), the Huon Estuary Study (CSIRO Huon Estuary Study Team 2000) and the Moreton Bay Study (Dennison and Abal 1999) have provided valuable information to managers and have resulted in better management.

In addition to understanding more about the systems we manage, it is also important to measure the performance or outcomes of management decisions and practices. What is the environmental dollar value for an artificial wetland versus more river bank fencing? This can be achieved by undertaking well-designed, hypothesis-based, monitoring programs.

1.2 WATER QUALITY, NUTRIENTS AND ENVIRONMENTAL IMPACTS

The major limiting nutrients for phytoplankton are nitrogen – in the form of ammonium (NH_4^+), nitrite (NO_2^-) and nitrate (NO_3^-) – and phosphate (PO_4^-). Nitrogen tends to be the limiting nutrient in marine systems, while phosphate is the limiting nutrient in freshwater systems. Nitrogen and phosphorous are needed for cell membranes and for proteins such as enzymes. These two nutrients are therefore of prime importance in water quality, and also

because human activities usually enhance their concentrations via sewage discharges, land clearing, excessive fertilisers and agriculture. Ammonia in particular is indicative of ‘new’ nutrient from human or animal sewage, while nitrate is indicative of ‘old’ or ‘aged’ nutrients from oceanographic upwelling. In high concentrations, ammonium is very toxic to plankton and fish. In low concentrations the less toxic ammonium chemically predominates, which is more easily assimilated by phytoplankton than nitrate. Two other nutrients – silica (Si) and iron (Fe) – are also limiting nutrients for some phytoplankton and are usually derived from the natural weathering of land. Therefore a useful benchmark is the ratio of N:Si or P:Si, which is used as a measure of human: natural nutrient sources.

The optimal proportion of nitrogen: silica: phosphorous for phytoplankton is 16:16:1, which is known as the Redfield ratio. Sewage and excessive use of fertilisers significantly alter this ratio, as well as altering the natural species composition of phytoplankton. Therefore not only the concentration of nutrients but also any changes to the ratio of abundance can increase the predominance of a single species – and some phytoplankton may begin to produce toxins under altered nutrient ratios.

Water quality and the extent of eutrophication have been assessed for decades by many management authorities from the analysis of water samples for nutrients and chlorophyll content. Such analyses are expensive and quality control of the chemical analysis and the sampling design has often been inadequate. Compared with oceanographic sampling, nutrients in enclosed waters vary rapidly over time, requiring collections particularly around rainfall events and with adequate replication. Nutrients may

BOX 1.2 EUTROPHICATION AND THE EFFECTS OF EXCESS NITROGEN

Compared with phytoplankton, seagrass growth needs less nitrogen relative to carbon to manufacture cellulose needed for structural support. Phytoplankton – and the algae that grows on seagrass – requires proportionally more nitrogen as their cells have little structural support. Consequently seagrasses thrive in clear, low-nutrient waters and can out-compete algae, taking up the sparse nutrients. When humans release nutrients into waterways, phytoplankton are no longer constrained and begin to shade the seagrass, and algae begin to grow on the seagrass blades. This results in a downward spiral – because with slower growth the seagrass blades become further covered in algae, which further retards their growth and encourages die-back, exposing the sediments and releasing more nutrients. Seagrasses are a useful indicator species of water quality, but they provide only a few years warning of an impending crisis.

behave in chemically and biologically complex ways – for example, algae may take up nutrients within hours and simply sequester them, waiting for warmer temperatures. Nutrient samples require stringent conditions for collection (such as wearing rubber gloves and controls to allow for the effect of boat exhaust) and laboratory analyses require particular attention to quality control. In general, many managers and scientists indicate that nutrient analyses provide a low value for the environmental dollar, and do not achieve the managers' aims. The frequency and spatial replication that water samples should be collected usually exceeds existing budgets.

Many water quality agencies are now in a position to assess their historical data, and find that it is not adequate to determine if water quality has declined in recent years. There is now the added effect of climate change on urbanisation of waterways and on water quality (Box 1.3). Some studies have failed not through lack of funds, but by a sampling design that did not have adequate controls or replication. Investment in unreplicated estuarine samples at regular monthly intervals would be better served by concentrating the same sampling effort at replicated sites during the summer and around rainfall events (see Chapter 4). In addition to wasted resources, poor water quality monitoring approaches make it difficult to report meaningful information to the community who use the waterway and who may be involved in the management of the system.

BOX 1.3 CLIMATE CHANGE

Plankton communities integrate various human and environmental inputs, thereby providing a benchmark for monitoring the synergistic effects of urbanisation and climate change (Kunz and Richardson 2006; Richardson and Kunz 2006). With shorter winters and longer summers, the seasonality (or 'phenology') of plankton and fisheries will each change, but not in the same way. Our collective challenge is to determine what the shifts in plankton communities imply. Or, in the parlance of climate scientists: what adaptations by plankton will affect the marine environment, from water quality to fisheries? The approximate doubling of atmospheric CO_2 expected by 2100 will increase ocean acidity (decrease pH), which will decrease the efficiency for plankton to form calcareous shells. Already it has been established that the shells of planktonic snails such as pteropods – with the more sensitive aragonite form of calcium carbonate – are affected by projected increases in carbon dioxide. The implications are enormous, because our biodiversity and fisheries are often derived from larvae with calcareous shells. There is no doubt that plankton communities will somehow change and adapt to warmer and more acidic oceans – we need to predict how human communities could also adapt.

It is better to select criteria that integrate with other environmental variables and conditions over a timeframe that matches the human timeframe and that provides better information about the system in question. Plankton is an effective integrator of temperature and nutrients over 3–7 days, thus providing useful information about the responses of a particular system. Plankton may not always be appropriate indicators and should only be used in accordance with specific objectives and sensible timeframes.

1.3 MANAGEMENT PLANS AND SAMPLING FOR A PURPOSE

A plankton study requires careful thought before field work starts. You need to consider:

- What does our organisation need to know about water quality, and how can this be achieved most cost effectively?
- Do we need a pilot study?
- Are there some trouble-spots (phytoplankton blooms, rainfall events)?
- Where should we establish a reserve and where should we allow fishing or bait collection?
- What about the future – should a monitoring program be in place, and for how long?
- What sites have been sampled in the past? Which could be re-visited?
- Is day or night sampling best for your purpose?
- Will our sampling program be able to detect a human impact against a naturally variable system?
- To what degree do samples need to be sorted? To type, family or species?
- Are other tests required (such as toxins)?
- How will you determine if change is due to natural variability or changes in catchment management?

Clearly there are multiple issues to be resolved before collecting samples. The degree of sorting the sample needs to be understood because the cost of collecting the samples will probably be less than a third of the total budget. Long-term data provides an important baseline against which future changes can be assessed. A general monitoring program should be conservative and easily interpreted by all, without relying on a single individual to execute. Good monitoring should also discriminate natural changes at control sites, as well as changes caused by humans. Otherwise potential developers may use this natural variation to hide any environmental impact that they may have caused.

Management plans need to be responsive to changes in conditions, requirements and available knowledge – and to their effectiveness. The key to adaptive management is to have a plan that is based on scientific knowledge and supported by a well-designed, monitoring program. However, the performance of many management actions is usually not monitored, and thus is not adaptive. It is very difficult to maximise environmental outcomes in proportion to investment if the outcomes are not monitored. Anecdotal evidence is not sufficient to determine the success of management actions. In order to ensure good science that supports management, the following hallmarks should be considered (see Table 1.1).

Table 1.1. Hallmarks of adaptive management plans.

Hallmark	Good practice	Poor practice
1. Objectives should be clear and unambiguous, linked to a timeframe such that their performance can be assessed.	Within 10 years we wish to have fencing and establish 10 m wide riparian vegetation belts between this tributary and a particular bridge.	Monitoring to satisfy public expectation that the water quality is not good enough.
2. Establish testable hypotheses, within the context of your sampling design and known variability.	By investing in fencing, the frequency of algal blooms (or percentage seagrass fouling, or water clarity) should significantly improve from 1990 levels.	The dairies and piggeries are responsible for the eutrophication in the estuary [an ambit claim].
3. Select suitable indicators that will respond to your management plan	The ratio of diatoms: dinoflagellates will increase to reference site levels after we implement this management plan. Or, we propose that a 20% increase in suspended sediment is a trigger for action (based on some credible study).	The plankton and biodiversity will improve after we implement this management plan [no quantitative measure of improvement].
4. Sampling locations should include reference or control locations	To test the environmental worth of the new sewage treatment plant, we will sample before, during and after construction, as well as in neighbouring embayments and an adjacent estuary	We will sample before, during and after we build artificial wetlands at the stormwater discharge [no study of neighbouring sites or estuaries to provide a context for the comparison].
5. Data interpretation and reporting	The number of replicate samples was in proportion to the sample-to-sample variability, and in proportion to the magnitude of change (see Section 4.2 for what constitutes a replicate sample).	There was a significant change in water-quality parameters before versus after construction [what level of change is required?]

1. Objectives should be clearly stated in the management plan in a manner that makes them transportable to specific monitoring programs. Management objectives should be measurable and linked to timeframes – providing a basis to assess their success. Any monitoring that is not linked to clear objectives can be considered a waste of resources because it is difficult to translate the results to management.
2. Testable hypotheses should be proposed, which must be matched by an appropriate sampling design. The statistical comparisons – in the light of the expected variance from a pilot study – should be peer reviewed to ensure that they are suitable for your study.
3. What indicators will be used to address each issue/hypothesis? What are the time frames within which data should be collected? Seek up-to-date guidelines and advice from experts. Good management plans include suitable performance indicators for each proposed management action. As with any adaptive management plan, management actions and their accompanying performance indicators should be reviewed over time and changed if necessary. Conceptual models of the system and the various functions taking place within the system can be useful when determining appropriate indicators for specific actions (Dennison and Abal 1999). Ensure that indicators are selected according to the needs and objectives of your particular management plan and not to suit the objectives of the authors of the particular text.
4. To determine whether any changes are the result of management intervention or natural variability, it is necessary to collect reference information from a number of control sites. The selection of appropriate control locations is a critical component of monitoring and expert advice should be sought. An increase in the frequency of algal bloom reports may be a natural phenomenon affecting a number of regions simultaneously, rather than the result of a local impact. External reference sites (for example, in collaboration with another council) are essential.
5. Results should be used to test your hypotheses and to assess whether your objectives have been fulfilled. Data can be used to provide advice about the degree of success of management actions in achieving objectives. Results of analyses and the interpretation of results should be clearly reported and linked to the objectives that they were assessing. There are a number of actions and outcomes of monitoring. Management actions may need to be changed for

objectives to be reached. This may entail researching other methods available to fulfil objectives. Any reports that result from monitoring activities should be peer reviewed.

The steps listed above may appear elaborate and may be expensive to undertake. There are, however, ways in which costs can be reduced. These include integrating monitoring programs among councils and government agencies, sharing control sites and working together with university groups. By working with experts, it is also possible to design studies that may not be overly expensive, but which can fulfill the objectives of the monitoring study. If monitoring is deemed too expensive to undertake appropriately, perhaps the management action should not be carried out as its success can never be quantified.

1.4 COASTAL ZONE MANAGEMENT

The coastal zone of a particular region generally consists of coastal waters out to 2 km offshore and to a particular distance inland. Precise definitions vary from place to place and it is important to ensure that before undertaking any management activities in the coastal zone that you obtain this information. Generally, however, the coast includes estuaries, coastal lakes, headlands, dunes, beaches, reefs, surf zones and open water.

Coastal zone management is considered separately from other forms of natural resource management because the coast is a special place that is under threat from a variety of natural and unnatural pressures. Effective management of the coast includes consideration of the pressures that cause problems, the issues that result from particular pressures, and an awareness of the impact that biophysical issues and pressures have on the broader community (social and economic effects). This is important because it is often the social and economic effects of an issue that are the real problem that needs to be tackled – and these effects should not be considered in isolation. For example, toxic algal blooms may result in the closure of oyster leases and a prohibition on fishing and recreational use of a particular waterway. This may deter holiday-makers from coming to the area, which could have ramifications on the rental market, the restaurant trade and other tourism-related industries. These, in turn, affect others in the community.

To effectively manage the coast, therefore, it is important to form a group of people who represent the interests of the local community and

other stakeholders. The composition of this management group (committee) is important, because they will not succeed if they are not considered to be representative.

The group must:

- determine the scale of the area they intend to manage. This can consist of a small area, such as an embayment, or a large stretch of coastline.
- establish broad goals of what they want to achieve in the long, medium and short term
- pull all available information together to develop a conceptual understanding of how the system of interest functions. Understanding the pressures on the system, and way the system responds to these pressures, is also important. It is essential that this understanding is not limited to biophysical understanding and includes social and economic information.
- use conceptual understanding to identify key knowledge gaps and seek to fill these knowledge gaps wherever possible
- establish management objectives, which should be clearly articulated, unambiguous and, where possible, should be measurable to enable effective monitoring and evaluation to take place
- establish appropriate management activities to effectively reduce the pressures that are causing the problems. It is possible to directly influence the problems, but these are generally more expensive to conduct and have a shorter duration.
- establish appropriate performance indicators for each management action
- create partnerships needed to deliver actions and the costs of implementation
- draw up a management plan once appropriate management actions have been established, priorities decided upon and consultation has taken place. This should generally be a stand-alone document that provides a background to the management area, a synopsis of the available information, a description of pressures and the state of the system, clear management objectives, management actions, partnerships, costs, timeframes, monitoring and evaluation framework.
- ensure that feedback loops are established to make the plan adaptive and able to be changed if the desired management outcomes are not being achieved or if better information/techniques become available.

In recent years, some useful computer models of catchments, water quality and socio-economics have been developed, which allow the interested, but under-funded, group to examine the many environmental options. Some of these models – and their benefits and limitations – are reviewed in the final chapter.

1.5 OUTLINE OF THIS BOOK

This book draws together disparate literature into a single volume, to convey a modern, pragmatic approach to water quality and the study of plankton. We are writing for the non-specialist, particularly those concerned with the quality of waterways. The study of plankton is not a curiosity or a class exercise, but an integrative measure of water quality. We use management issues with examples and logistics to direct the content of this guide and to lead each chapter.

Plankton size is a persistent theme throughout this guide. It is the first feature that a novice can use and it is a pervasive feature in many plankton models of nutrient uptake, growth, longevity and grazing rates. We have used a consistent millimetre scale in our sketches, in relation to basic plankton collections and basic microscope optics. Few plankton keys are provided – instead we provide sketches as a guide to the common large types and, where possible, provide a reference to detailed guides.

Chapter 2 provides an overview of plankton habitats and ecology for the non-specialist.

Particular examples of water quality issues are provided in Chapter 3, which shows how solutions were provided through plankton studies. The plankton issues are structured around a problem for management. These water quality issues should be read before tackling the details of plankton in the subsequent chapters.

Chapter 4 covers how to sample plankton and the advantages and disadvantages of different types of sampling gear. We begin the chapter by giving examples of good and poor sampling designs. We provide an overview of some sampling designs that are necessary to detect environmental impact and change.

The next four chapters are the core of this book – providing a general guide to the major groups of plankton. An overview to identifying larger freshwater and marine phytoplankton is provided in Chapters 5 and 6, respectively. These larger phytoplankton can be observed with a drop of water sandwiched between a microscope slide and cover slip and using a basic compound microscope. We provide no simple guide as to whether a

particular cell is toxic (but see Hallegraeff 1991). Chapter 7 and 8 cover freshwater and marine zooplankton, respectively. We have taken a pragmatic approach to our guide: focussing on what someone who is new to working with plankton might notice and drawing on a number of useful local guides. In the final chapter, we return to the water-quality issue and provide an overview of useful models and other tools to study coastal and estuarine water quality.

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Chapter 2

Plankton processes and the environment

Anna M. Redden, Tsuyoshi Kobayashi, Iain Suthers, Lee Bowling, David Rissik and Gina Newton

2.1 PLANKTON ECOLOGY AND THE EFFECT OF SIZE

For plankton communities, size really does matter! Individual members of the plankton vary greatly in body size: ranging from minute viruses and bacteria, to the microscopically visible phytoplankton and small invertebrate larvae, to the large gelatinous zooplankton (jellyfish). In fact, planktonic organisms span seven orders of magnitude in length: from 0.2 micrometres to about 2 metres. A micrometre (µm), or 'micron', is a thousandth of a millimetre, that is, 1 µm = 0.001 mm. A human hair is about 10 µm thick (100 hairs = 1 mm); the standard pin used to package shirts is about 600 µm (0.6 mm) thick; and a dissecting needle used in many science classes as a plankton probe is about 1 mm thick. It will be useful for you to check these dimensions using a microscope and ruler as your microscopic benchmarks – particularly for zooplankton. The resolution of the best light microscopes is about 0.5 µm – of course, electron microscopes are much better than that (0.2 nanometres with a transmission electron microscope; Kane and Sternheim 1978).

As there are significant ecological and physiological implications of body size in plankton (Peters 1983), we use plankton size as a first step in classification.

The various size categories of plankton are as follows:

- **megaplankton** are those large floating organisms that exceed 20 cm in length. They are represented by very large jellyfish, salps and their relatives
- **macroplankton** (2–20 cm, Figure 2.1 top) include large visible organisms such as krill, arrow worms, comb jellies and jellyfish
- **mesoplankton** (0.2–20 mm, Figure 2.1 bottom) are very common and visible to the naked eye; they are diverse and include copepods, cladocerans, small salps, the larvae of many benthic organisms and fish, and others
- **microplankton** (20–200 μm, Figure 2.2 top) include large phytoplankton (large single-celled or chain-forming diatoms, dinoflagellates), foraminiferans, ciliates, nauplii (early stages of crustaceans such as copepods and barnacles), and others
- **nanoplankton** (2–20 μm, Figure 2.2 bottom) include small phytoplankton (mostly single-celled diatoms), flagellates (both photosynthetic and heterotrophic), small ciliates, radiolarians, coccolithophorids and others
- **picoplankton** (0.2–2 μm) are mostly bacteria (called bacterioplankton). They require at least 400× magnification for detection and counting. Marine viruses are even smaller (less than 0.2 μm).

The size categories listed above do not reflect particular taxonomic divisions as sizes vary greatly within most taxonomic groups. In addition, size does not reflect any trophic classification. Small plankton may include photosynthetic cells (that is, autotrophs or 'self-feeders'), herbivores, carnivores or omnivores (that is, heterotrophs like us). Many phytoplankton cells maintain hundreds of other small symbiotic cells around them, sometimes for their nitrogen fixation (such as by blue-green algae). Some organisms even maintain symbiotic relationships with photosynthetically active cells known as zooxanthellae (as in many corals, sea anemones, sponges and clams of tropical coral reefs). Large plankton, such as some jellyfish, are akin to carnivorous plants – capturing copepods and small fish for their nitrogen.

Cell size has direct consequences for many physiological processes, including the assimilation of dissolved nutrients from the environment. Up until the 1970s, the importance of picoplankton (cell size: 0.2–2 μm), relative to the larger nano- and microphytoplankton, such as diatoms and dinoflagellates,

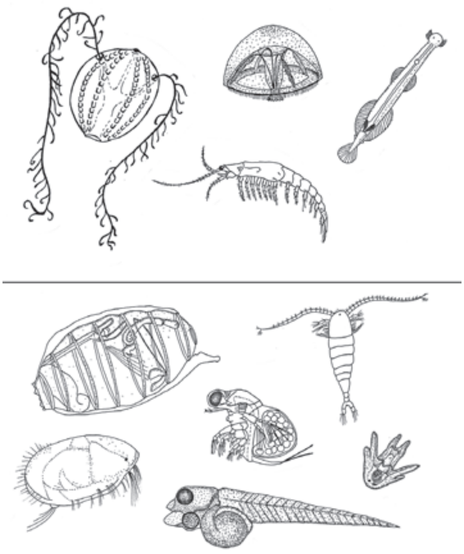


Figure 2.1 Examples of some typical members of the macroplankton (2–20 cm, top panel, from left to right: ctenophore, krill, jellyfish, arrow worm) and mesozooplankton (0.2–20 mm, bottom panel, left to right: ostracod, salp, larval fish, cladoceran, copepod, pluteus larva of a sea urchin).

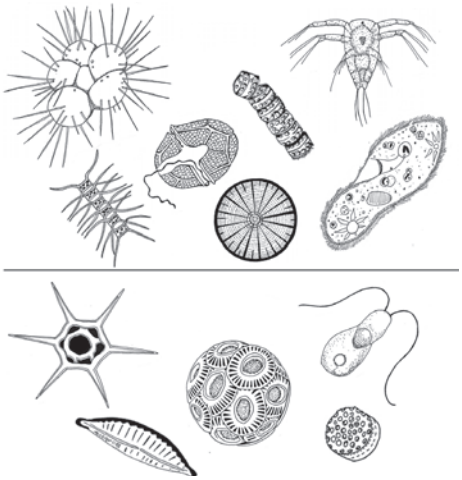


Figure 2.2 Examples of some typical microplankton types (20–200 μm, top panel, left to right: radiolarian, diatom chain, armoured dinoflagellate, centric diatom, dinoflagellate chain, nauplius (larval crustacean), ciliate) and nano-plankton types (2–20 μm, bottom panel, left to right: silicoflagellate, pennate diatom, coccolithophore, flagellate, diatom).

was largely unrecognised. We now know that these tiny cells, which are about the size of bacteria, can dominate the phytoplankton – contributing up to half the chlorophyll-*a* content in coastal waters, and up to 90% in nutrient-poor oceanic waters, and producing much of the oxygen we breathe.

Low-nutrient (oligotrophic) waters are typically dominated by small phytoplankton cells, which are much more efficient at using small amounts of available nutrients than are large cells. Small phytoplankton have a competitive advantage under low-nutrient conditions because they have a higher cell surface area: volume ratio than large phytoplankton with which to take up available nutrients across their cell membrane. For the most part, large phytoplankton cells appear in abundance primarily in response to periodic nutrient increases (for example, seasonal rain events) and/or localised inputs. Other features of plankton that are related in some non-linear way with size are growth, carbon content, sinking rates, grazing, swimming, fecundity and longevity (Peters 1983; Baird and Suthers 2007).

2.2 PLANKTON FOOD WEBS

The most important elements for phytoplankton growth are the macronutrients nitrogen (N) and phosphorous (P) and, for diatoms, silica (Si). Phytoplankton cells take up dissolved forms of C, N and P across their cell surfaces in an atomic ratio of 106C:16N:1P (the Redfield ratio). Sometimes the atomic ratios of dissolved nutrients in the water column are different to those required for phytoplankton growth. This provides an important signal to managers and researchers. N: P atomic ratios that are much higher than 16 (say, 25–30) suggest that P limitation of algal growth is occurring, which means that the lack of phosphorous is preventing further algal growth. Alternatively, a ratio of less than 10 would imply N-limited growth.

While phytoplankton growth in freshwater systems is generally P limited, growth in estuarine and oceanic environments is commonly N (and at times also Si) limited. Phytoplankton cells require external sources of other inorganic nutrients, in particular trace metals and minerals (Fe, Mg, Zn, Na, Ca, Mn and others) and vitamins (thiamine, biotin and B_{12}). These are needed in much lesser quantities and are generally assumed (wrongly at times) to be in sufficient quantities for growth.

In some regions of the world's oceans, phytoplankton cells have access to relatively high levels of N and P yet exhibit low biomass (generally determined by chlorophyll-*a* concentration). A series of elaborate experiments in the Equatorial Pacific demonstrated that this 'high-nutrient, low-biomass' phenomenon was due to iron limitation (Behrenfeld *et al*. 1996, Timmermann *et al*. 1998).

In areas of low phytoplankton productivity, most of the phytoplankton growth is sustained through 'regeneration' of nutrients. This happens when organic matter (for example, faecal pellets and dead and decaying material) is remineralised to dissolved inorganic nutrients via microbes in the plankton. 'New' production occurs in response to external nutrient inputs (catchments, rivers, atmosphere, and so on) or when turbulent diffusion allows deep water nutrients to cross the thermocline (nutricline) into the surface mixed layer. The ratio of new to regenerated production is referred to as the *f* ratio – the lower the *f* ratio, the greater the dependence on regeneration of nutrients via microbes. Although used as an index of trophic status of an area, the *f* ratio can vary greatly over time (Platt *et al.* 1992).

Grazers represent an essential trophic pathway for the transfer of organic carbon from phytoplankton to fish, and they contribute to the nutrient pool by excreting faecal pellets that are either recycled within the water column or used by bottom feeders. Nutrient recycling is also assisted by the 'sloppy feeding' or partial ingestion of cells by herbivorous zooplankters (such as copepods), which results in the release of nutrient-rich cell sap following handling and rupture of captured cells.

Trophic transfer, however, is no longer understood simply as materials and energy passing through producers and a series of consumers in a simple linear chain (the classical food chain). The traditional model of a short marine food chain (phytoplankton → copepod → fish) became obsolete following recognition of the trophic importance of bacterioplankton and protozoans in marine waters (Malone 1971; Williams 1981). It is now accepted that a significant proportion of phytoplankton production is not consumed directly by zooplankton grazers, but is cycled by the microbial community ('microbial loop') before it becomes available to consumers.

The primary organisms involved in the recycling activities of the microbial loop (Figure 2.3) are water-column bacteria, heterotrophic flagellates and ciliates. One of the roles of the bacteria is to break down organic molecules contained in non-living particulate organic matter (POM) and dissolved organic matter (DOM) derived from living cells, faecal pellets and dead and decomposing bodies. The bacteria convert organic matter to dissolved inorganic nutrients (DIN), such as nitrogen, phosphorus and potassium, which are then available for rapid uptake by phytoplankton. The bacteria are consumed by protozoans (ciliates and nano-flagellates), which are in turn food sources for other zooplankton.

The recycling of POM by the microbial loop also serves to reduce the sedimentation of faecal matter and detritus. This is particularly important in warm, low-nutrient waters, where microbes rapidly and efficiently recycle materials and thus limit the sinking of large amounts of organic matter to

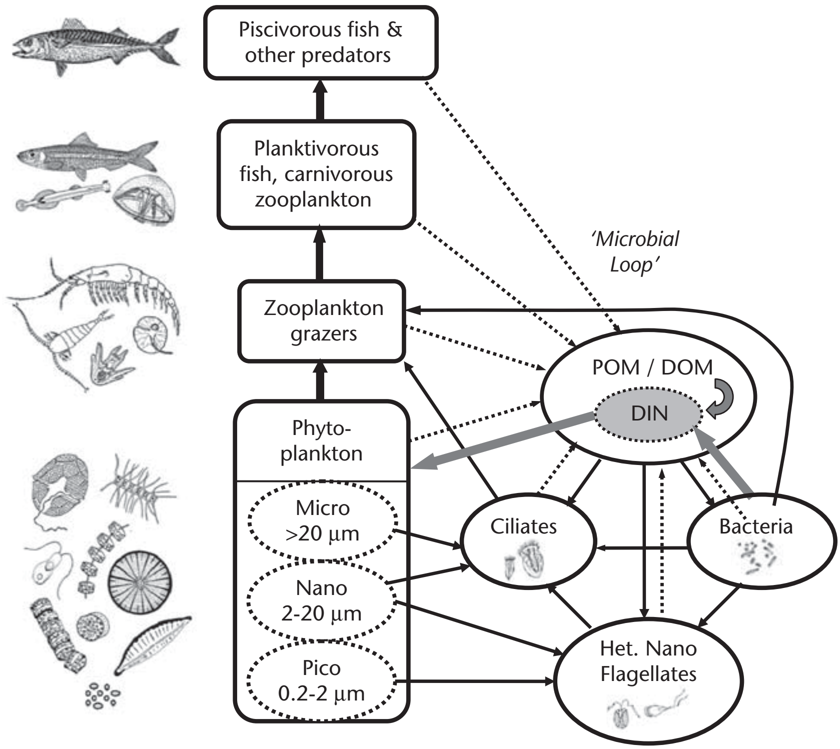


Figure 2.3 Generalised food web showing classical food chain (left side) and microbial loop (right side), with arrows showing trophic pathways, flow of particulate and dissolved organic matter (POM, DOM) in excretory products and dead organisms (dashed arrows), and flow of dissolved inorganic nutrients (DIN) to phytoplankton. Het. = Heterotrophic.

the bottom. In cold waters – and during the winter months in many temperate regions – microbial activity is suppressed. The effects are that most of the carbon reaches higher trophic levels directly via the grazing activities of zooplankton, and a large fraction of the carbon fixed during photosynthesis sinks to the bottom where it is then used by benthic communities.

Numerous feeding strategies are employed by small zooplankton (ciliates and flagellates) including herbivory, carnivory and omnivory. But a strategy commonly used by many is 'mixotrophy' – a feeding strategy that combines characteristics of both autotrophs (which make their own food via photosynthesis) and heterotrophs (which ingest food). Numerous species of ciliates that are known to exhibit mixotrophy contain large numbers of chloroplasts (light-harvesting organelles) sequestered from ingested

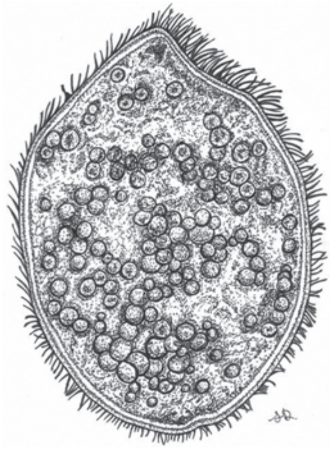


Figure 2.4 Mixotrophic ciliate with numerous chloroplasts (organelles containing light-harvesting pigments) sequestered from ingested algal cells. (Cell diameter 10–20 μm.)

phytoplankton (Figure 2.4). They derive nutrition from both the direct ingestion of food and by the carbohydrates made by the sequestered photosynthetically active chloroplasts (Stoecker 1987). This nutritional strategy offers great survival and competitive advantages, especially in environments where food resources are highly variable.

2.3 PLANKTON BEHAVIOUR: SINKING, BUOYANCY AND VERTICAL MIGRATION

Cell size has a significant impact on the ability of phytoplankton cells to maintain their position at depths with adequate light and nutrients to sustain growth. In general, an increase in cell size results in an increase in sinking rate – with dead cells sinking at faster rates than live cells. Large phytoplankton cells (such as diatoms) are disadvantaged by being highly susceptible to sinking, and may require strong vertical mixing (for example, caused by upwelling or strong winds) to maintain their position in surface waters.

Sinking of cells can be reduced by morphological structures that increase cell, or colony, resistance to sinking. The flagella of many nanoflagellates serve, in part, to overcome sinking. Adaptations of large and heavy cells (large diatoms and dinoflagellates) to reduce sinking, and to maintain near neutral buoyancy and vertical position in the euphotic zone, include chain formation and cell extensions that provide a high surface area: volume ratio. Cell extensions can be highly numerous and include protuberances, spines,

horns, wings and hair-like structures. They increase frictional drag and also increase the effective size of phytoplankton cells, which makes them more difficult for zooplankton grazers to capture and ingest. Another advantage of cell extensions – particularly diatom spines – is that they can house large numbers of chloroplasts and thus increase the ability of cells to harvest light for photosynthesis.

Cell density, and thus rate of sinking, is also affected by the composition of cells. Silica-laden diatoms are particularly heavy. Mechanisms to control cell density, and thus location within the water column, may include production of gas vacuoles and the accumulation of fats and oils, which are lighter than water. Cell aging and nutritional state of phytoplankton cells are physiological conditions that affect cell density. Post-bloom nutrient-starved diatoms tend to sink significantly faster than nutrient-rich diatoms (Tilman and Kilham 1976). This effect is frequently demonstrated in temperate and polar waters, where mass sinking of phytoplankton blooms occurs following nutrient exhaustion. A large proportion of bloom material may settle to the bottom as diatom flocs or aggregates (>0.5 mm) composed of algal cells, zooplankton remains, faecal pellets and other forms of detritus. These highly visible settling flocs are commonly referred to as 'marine snow'.

Zooplankton features that increase drag, and thus reduce sinking, include long, thin or flattened body shapes, and projections such as hairs, long spines and wings. Buoyancy may also be assisted by small droplets of oil. Many planktonic animals can swim reasonably well, or are able to control their position by selecting different depths and currents, or by adjusting buoyancy. Many species of crustacean zooplankton – especially the adult forms – are strong swimmers and conduct diel vertical migrations through the water column (Figure 2.5). This involves rising to surface waters at dusk and grazing heavily on phytoplankton cells throughout the night, before descending to deeper waters well before dawn (although some interesting cases of reverse migrations are known: that is, rising up in the day, and dropping back down at night). The distance travelled during diel vertical migration can range from a very short distance (less than 2 metres in coastal lagoons) to hundreds of metres up and down in 24 hours in oceanic waters).

Diel migratory behaviour is triggered by changes in light intensity, and is largely an adaptation to avoid visually feeding predators, particularly fish. Migratory patterns can be variable, and are known to differ with the sex and age of the species, habitat type and season (van Gool and Ringelberg 1998). Many gelatinous plankton (such as jellyfish) and larval crustaceans (such as prawns) exhibit tidal-driven vertical migrations into estuaries. They move

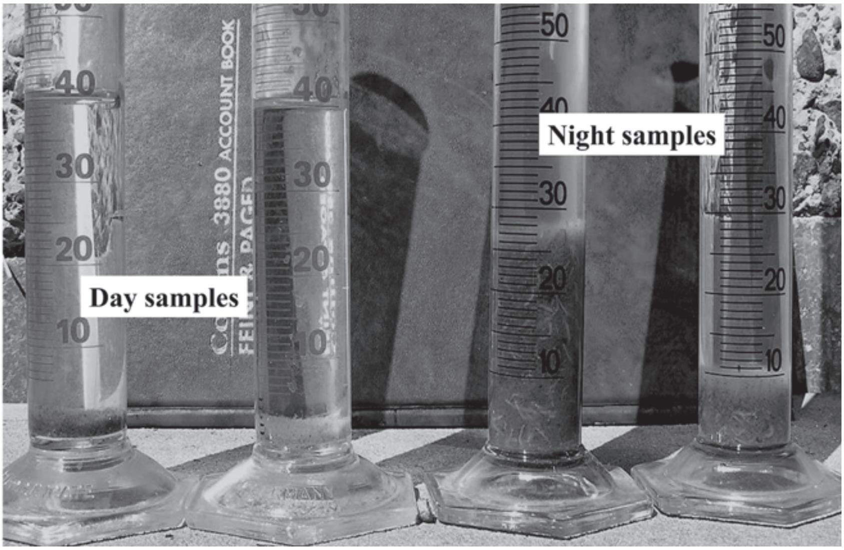


Figure 2.5 Representative catches of zooplankton during the day (two on the left, with <1 mm displacement volume), and during the night (two on the right, with 22 and 10 mm displacement volume). In some years there may be no difference between day and night zooplankton abundance.

up into the flood tide waters – especially at night – and are transported into the estuary, and move lower in the water column during ebb tides to avoid being carried out. Such migrations are entrained into the circadian rhythm of many organisms, such that some diel and tidal activities continue to be observed even after the organisms are removed from their natural environment (for example, when maintained in a laboratory).

2.4 LIFE CYCLES OF ZOOPLANKTON

In general, the smallest plankton have the shortest life cycles: bacteria and flagellates generally multiply within a few hours to one day. Most mesozooplankton have life cycles of a few weeks, while the macro- and megaplankton usually have life cycles spanning many months and longer.

Many zooplankton spend their entire life cycle as part of the plankton (for example, copepods, salps and some jellyfish) and are called holoplankton. The meroplankton, which are seasonally abundant, especially in coastal waters, are only planktonic for part of their lives (usually at the larval stage). Most bear little, if any, resemblance to the adult form and drift for days to weeks before they metamorphose and assume benthic

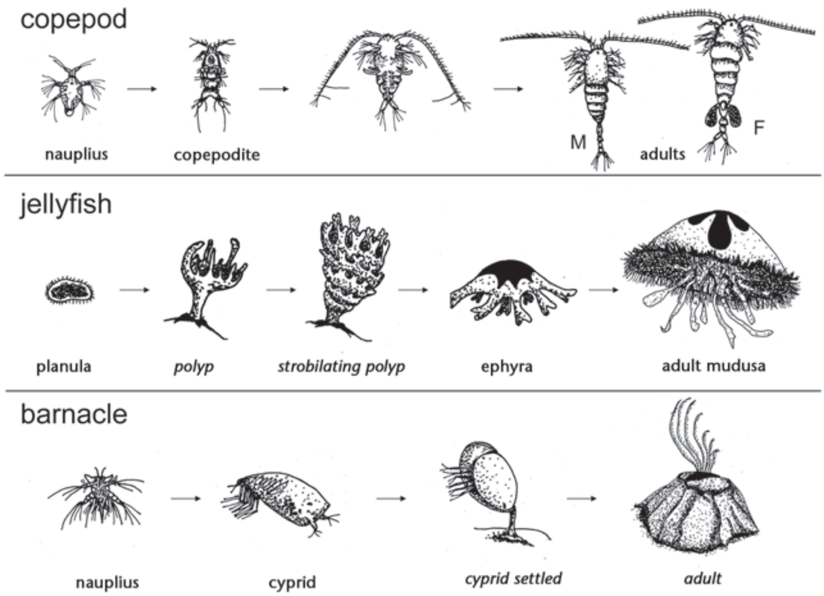


Figure 2.6 Life stages (larval to adult form) of a typical copepod, barnacle and jellyfish. Names in italics refer to those life stages that are not planktonic, when the animal becomes attached to hard surfaces.

or nekton lifestyles. Examples of meroplankton include the larvae of sea urchins, starfish, crustaceans, marine worms and most fish. Planktonic and sessile life stages of some common zooplankton types are shown in Figure 2.6 and are described below.

The general copepod life cycle includes six nauplius stages (larvae) and five copepodid stages (juveniles) prior to becoming an adult. Each stage is separated by a moult and, as the stages progress, the trunk of the copepod develops segmentation. Sexes are separate, sperm is transferred in a spermatophore from the male to the female, and eggs are either enclosed in a sac until ready to hatch or released as they are produced. Development times from egg to adult are typically in the order of 2 to 6 weeks, and are significantly affected by temperature and food availability. The life-span of adults may be from one to several months.

Barnacles also have free-swimming nauplius stages, followed by a carapace-covered cyprid stage after the final naupliar moult. Cyprid larvae are attracted to settle on hard substrates by the presence of other barnacles, ensuring settlement in areas suitable for barnacle survival and for obtaining future mates. After settling, the cyprid releases a substance to permanently

> **BOX 2.1 PLANKTON DIVERSITY**
> In 1961, the great biologist GE Hutchinson wrote a speculative essay entitled 'The paradox of the plankton', expressing surprise at the high diversity of plankton in an otherwise fairly uniform environment (Hutchinson 1961). Classical competition theory would suggest that, without disturbance, there should be very low diversity – particularly for holoplankton. The key is that the ocean environment is not uniform, but is divided into characteristic water masses, and is not without disturbance caused by seasonal changes and storms. Modelling also suggests high diversity is possible when there are hundreds of species (rather than tens of species), each with their own life cycles, sizes and physiology.

cement itself to the substrate. Calcareous plates then grow and surround the body. The appendages face upwards to form cirri which sweep food particles into the organism. The adults are hermaphroditic (each with both male and female parts) and reproduce sexually by cross fertilisation. The adult broods the fertilised eggs within the shell until they develop into nauplius larvae. Over 10 000 larvae may be released by a single adult.

Life cycles of jellyfish are complex, with generally two adult morphologies: polyp and medusa (typical jellyfish form). The sexes are separate and mature adult medusae release eggs and sperm, which, upon fertilisation, form free-swimming, hair-covered larvae known as planulae. After a few days to weeks, the planulae settle on hard substrates and metamorphose into tiny sessile polyps (which look like upside-down jellyfish), which clone themselves and bud (strobilate). Juvenile jellyfish (ephyrae) peel off from the stack, float into the plankton as young jellies and grow into adult medusae. This transformation can take a few weeks up to a few years, depending on the species of jellyfish.

2.5 FRESHWATER HABITATS OF PLANKTON

There is a wide variety of inland aquatic systems within Australia – ranging from rivers and streams to lakes and reservoirs, farm dams and ponds, billabongs and wetlands (Figure 2.7). Due to low rainfall and high evaporation in many parts of the country, there is often a scarcity of permanent water bodies. Rivers and streams are often ephemeral – containing flowing water only after rainfall. Natural lakes are rare – reservoirs built to conserve water for town water supply and for irrigation are more common.

Inland waters – as distinct from estuarine or marine environments – are often considered to be fresh, with low concentrations of dissolved salts.

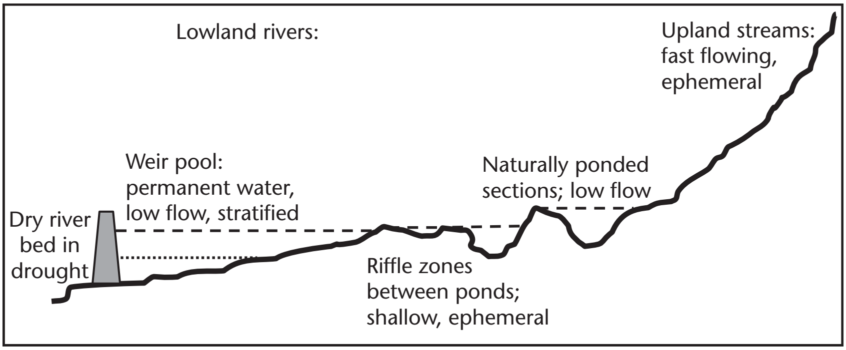


Figure 2.7 Diagram of a stream network and pool formation as phytoplankton habitat. Upland streams provide an input of nutrients, but are poor phytoplankton habitat. The naturally ponded sections have a reduced flow rate, which allows water residence times to match cell doubling times. Riffle zones can provide habitat for benthic forms. The weir provides permanent water, but can become stratified and de-oxygenated.

However, salt lakes may have salinities greater than that of sea water. Williams (1980), in arbitrary terms, defined fresh water as that with a salinity of less than 3 grams per litre of dissolved salts. In lowland areas with low rainfall and high evaporation, the salts of inland waters are often dominated by sodium and chloride, rather like sea water. In upland headwater streams and reservoirs, the waters are much fresher and calcium and magnesium bicarbonates may be the predominant salts present.

Rivers and streams are the primary routes of catchment drainage. During flood events, rivers may break out of the confines of their river channel, with their waters then spreading out over the floodplain. On these occasions, they can also transport large quantities of sediment and nutrients downstream from the catchment. In contrast, during droughts stream flow in permanent rivers is sustained by drainage from adjacent groundwater systems, while many others cease flowing completely, with only isolated pools remaining. The characteristically shallow nature, steep gradients and high flow velocities of upland rivers and streams keeps their waters well mixed (Figure 2.7). Many of the larger Australian rivers are impounded behind dams as they emerge from highland areas. After exiting these areas many inland rivers, such as those within the Murray–Darling Basin, then traverse many hundreds of kilometres of flat, lowland country. Gradients are small and channels become broad and meandering, or split into anabranches and distributary channels – with many terminating in extensive wetland areas. Lowland rivers may be impounded in natural ponds or by constructed

weirs where water depth will increase, flow velocities will decrease and the resultant ponds and weir pools then assume more lake-like characteristics, including stratification of the water column during summer in some if they are deeper than 3 metres. Fine sediment washed in from the catchment make many of these water bodies turbid. Nutrient and light availability, rate of flow, and stratification will all affect plankton community composition and abundance in these rivers (Mitrovic *et al.* 2003).

Flowing river systems are generally not good habitats for plankton, because the organisms entrained within the water column are continually displaced downstream. However, some of the larger lowland rivers may develop their own riverine phytoplankton communities – known as potamoplankton – which develop within parcels of water as these traverse the length of the river. Most algal growth in smaller, shallower, faster flowing streams, however, is confined to clumps of filamentous algae attached to a secure substrate to prevent themselves from being washed away, and to films of microscopic algae coating the surfaces of rocks, mud, sticks and aquatic macrophytes. These algae obtain the substances they require to sustain their growth as the water flows over them. The weir pools and ponded sections of lowland rivers and streams may, however, become suitable habitats for phytoplankton to form blooms. Some rivers also have small embayments, inlets, or backwater areas where water movement may be minimal. These areas – known as 'dead zones' – are areas where phytoplankton can develop (Mitrovic *et al.* 2001).

Lakes, reservoirs, farm dams, ponds, billabongs and wetlands are characterised by prolonged residence times of the water they contain, and the limited mixing of water within them – apart from that caused by wind-driven currents and internal-heat-transfer processes. Deeper lakes and reservoirs undergo strong thermal stratification during the warmer months of the year, caused by the preferential solar heating of the surface waters. Water density decreases as temperature increases, so warm water overlies colder water and creates horizontal density gradients that resist vertical mixing and enhance the stability of the water column. Chemical and biological demand for oxygen in deeper regions, accompanied by limited replenishment from the surface due to the lack of vertical mixing, can lead to very low oxygen levels in deep lake waters. Deoxygenation of the deeper waters has major effects on the chemistry of other substances, especially nutrients, which can be mobilised from the lake sediments under such conditions. The thermal stratification and mixing regimes of lakes and reservoirs influences water column stability, nutrient availability and light availability at different times of the year – and, consequently, the plankton community structure and abundance in these water bodies.

> **BOX 2.2 CHANGING STATE OF A FRESHWATER LAKE**
> Lake Makoan in Victoria provides a good example of a reservoir that underwent a change of state: from a clear-water, macrophyte-dominated system, to a turbid, phytoplankton-dominated system. The lake dried out during droughts in the 1980s, the macrophytes died and the fine sediment on the lake bottom was exposed. This became suspended in the water column when the lake refilled. The water became very turbid, and light could not penetrate to the bottom for the macrophytes to re-establish. Instead, with high nutrient concentrations, cyanobacterial blooms took over.

The plankton of lakes has been termed limnoplankton, while that of ponds heleoplankton. While some species of phytoplankton may be characteristic of rivers, lakes or ponds, there are sufficient common species found in all three habitats that the classification of phytoplankton communities into these groupings has only very general application.

Farm dams are often very turbid environments, so lack of light within the water column may limit phytoplankton growth. These, and other small ponds, are often typified by high amounts of organic substances in the water, which is often thought to favour certain kinds of motile unicellular algae known as euglenoids (Chapter 5, Section 5.6). Wetlands and billabongs are generally shallow, and much of the submerged area may be occupied by aquatic macrophytes, especially angiosperms, but also by some large macroalgae, known as charophytes, that grow from the sediments. These macrophytes, and algae that grow attached to them (termed epiphytes) may compete with phytoplankton for light and nutrients, so that wetlands may not be good habitats for phytoplankton. Shallow water bodies may be clear water, macrophyte-dominated systems, or turbid, nutrient-enriched, phytoplankton-dominated systems (Scheffer 1998) (Box 2.2).

2.6 ESTUARINE AND COASTAL HABITATS OF PLANKTON

Estuary processes determine the fate of nutrients discharged from river catchments.

These processes include:

- physical dynamics (such as rainfall, water residence times and tidal flushing), catchment effects (including nutrient and sediment run-off)
- biological function (such as primary production by algae, whether they are benthic, phytoplankton or macro-algae and seagrass)

- biogeochemistry (where bacteria may shift nutrients, such as nitrogen or phosphates, from the sediment or into the air)
- factors such as secondary and tertiary production.

Traditionally, an estuary is defined in terms of the limit of penetration of oceanic salt, which moves upstream under the influence of the ocean tide. In this sense, a commonly used definition is that of Pritchard (1952), who defined an estuary as ‘a semi-enclosed coastal body of water that has a free connection with the open sea and within which sea water is measurably diluted with fresh water derived from land drainage’. However, this definition does not include lakes and lagoons that are often not influenced by tides.

A broader definition would take into account the diversity and spatial variability of estuarine fauna and flora. Collett and Hutchings (1977) define estuaries as the tidal portions of river mouths, bays and coastal lagoons, irrespective of whether they are dominated by hypersaline, marine or freshwater conditions. Included in this definition are inter-tidal wetlands – where water levels can vary in response to the tidal levels of the adjacent waterway – together with perched freshwater swamps, as well as coastal lagoons that are intermittently connected to the ocean.

The tidal range undergoes a regular fortnightly cycle, increasing to a maximum over a week (spring tides) and then decreasing to a minimum over the following week (neap tides), because of the monthly orbit of the moon around the earth. Solstice tides, or king tides occur in June and December of each year, when the sun is directly over the Tropics of Cancer and Capricorn, respectively.

The characteristics of tides vary across spatial scales. For example, on the south east coast of Australia, tides are generally semi-diurnal with high and low tides occurring about twice a day. These tides have diurnal inequality where the height of two consecutive tides varies (Figure 2.8). Tides elsewhere have different characteristics: for example, many regions in Western Australia experience one tidal cycle each day (a diurnal tide).

Inside the estuary, the timing and dynamics of tidal currents become more complicated. Meanders around topography can slow tidal movement upstream, such that peak tides upstream occur hours after peak tides on the coast. The tidal limit of an estuary is the region of an estuary where there are no discernable changes to water levels as a result of tidal movement. The salinity limit is where there are no measurable changes to salinity over tidal cycles. The tidal limits and saline limits are often different, with tidal limits generally being further upstream.

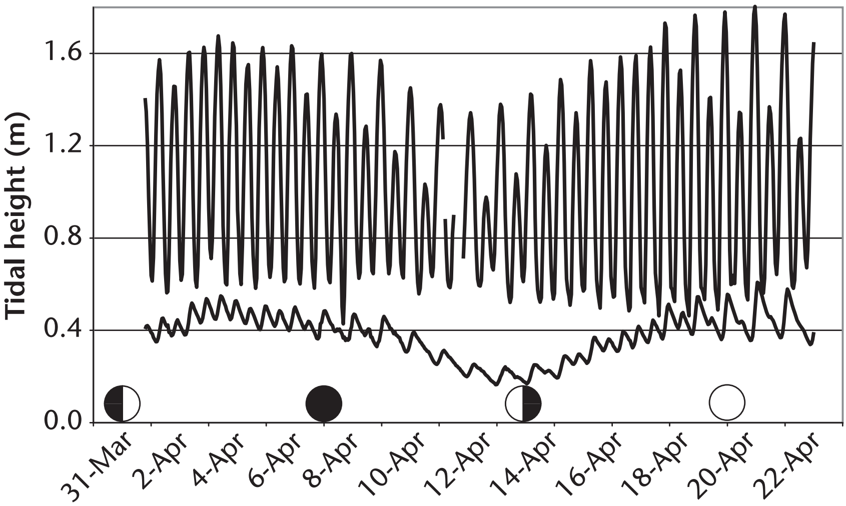


Figure 2.8 Progression of the tides within a day, and over a lunar month. The upper line shows tidal fluctuation on the open coast, while the lower line shows the damped tide inside a nearby coastal lagoon. (NSW DECC.)

Flood and ebb tides have different velocities, which can result in more water moving upstream into estuaries at flood tides than leaving at low tides. This can change the flow regimes of these systems (Figure 2.8).

The shapes of estuaries can influence the behaviour of tidal movement. In some estuaries with long thin channels upstream of a wide embayment near the ocean, the change of shape can force the upstream tidal range to be greater than that downstream. Alternatively, tidal movement becomes attenuated rapidly in estuaries with thin channels connecting them to the ocean, but which have wide reaches upstream. Influencing the depth or width of estuaries through dredging activities or by seawall construction can affect their hydrology.

Run-off from the land can vertically stratify the estuary, with less dense, brackish, turbid water on top and denser, salty, clear, oceanic water beneath. This salty layer is sometimes termed 'the salt wedge' and can penetrate many kilometres upstream, along the bottom (see Section 2.7). When there has been no recent downpour, one can place two floats in the estuary – one with a drogue near the surface and the other with a drogue just off the bottom – and observe the surface float move downstream and the bottom one move upstream.

In the coastal ocean, the surface waters are warmed by the sun and, along with wind mixing and some fresh water, to create a surface mixed

layer that may be 2 to 50 m deep. The layer may completely disappear during the winter storms, or become very shallow during hot calm days. The temperature boundary between the two layers is known as a thermocline. Other similar boundaries include haloclines (by salinity), pycnoclines (by density), or nutriclines (by nutrients). At the temperature boundary, phytoplankton find the best of light and nutrient conditions and frequently bloom – forming a sub-surface chlorophyll maximum.

Even a wind- and tidally mixed estuary is remarkably structured into different planktonic habitats. The most obvious is where the 'estuarine plume' of brown brackish water meets the clear blue ocean water. Within a matter of minutes, or metres, you could be sampling completely different water (Figure 2.9a). If you are not aware of this change, then your 'replicate' samples will be very different – making any comparisons very difficult. The estuarine plume is usually less dense by nature of lower salinity (even fractionally less), and is also identified by colour, and by being warmer in summer and cooler in winter than the ocean. An estuarine plume is usually quite shallow – less than a few metres deep (Figure 2.9b) – such that in the wake of a ship cutting across the plume one can see the clear ocean water churned up from beneath.

Where the 'brown meets the blue', there is a convergence where the denser ocean water wedges underneath the estuarine plume, leaving any buoyant material from either side trapped at the surface as an oily looking line of water, mixed with flotsam. This line is known as a slick, or a 'linear oceanographic feature' (Kingsford 1990). Not only are these slicks evident near the estuary mouth on the ebb tide, they are evident on the flood tide, often as a 'V-shaped' front (Figure 2.10). This is because the ocean water is retarded by the shore line, while the ocean water in the central channel can push further upstream. Both ebb tide and flood tide fronts are favourite haunts of seagulls and pelicans.

Other convergence lines are evident behind islands and headlands (for example, Suthers *et al*. 2004). It is thought that pre-settlement fish and invertebrates may be concentrated in these slicks, which are often moved onto reefs or seagrass beds as the tide turns. In this oceanographic way, some areas characteristically receive more young prawns and fish than other parts of estuaries and deserve to be protected (or rehabilitated). It is important to note that tidal wakes and eddies exist for up to 6 hours of a sinusoidal varying current, while the wake of an oceanic island can last for weeks (for example, Heywood *et al*. 1990; Suthers *et al*. 2006).

Islands in shallow water (less than 40 m deep) have different oceanographic processes to deep oceanic islands. The wakes of shallow islands

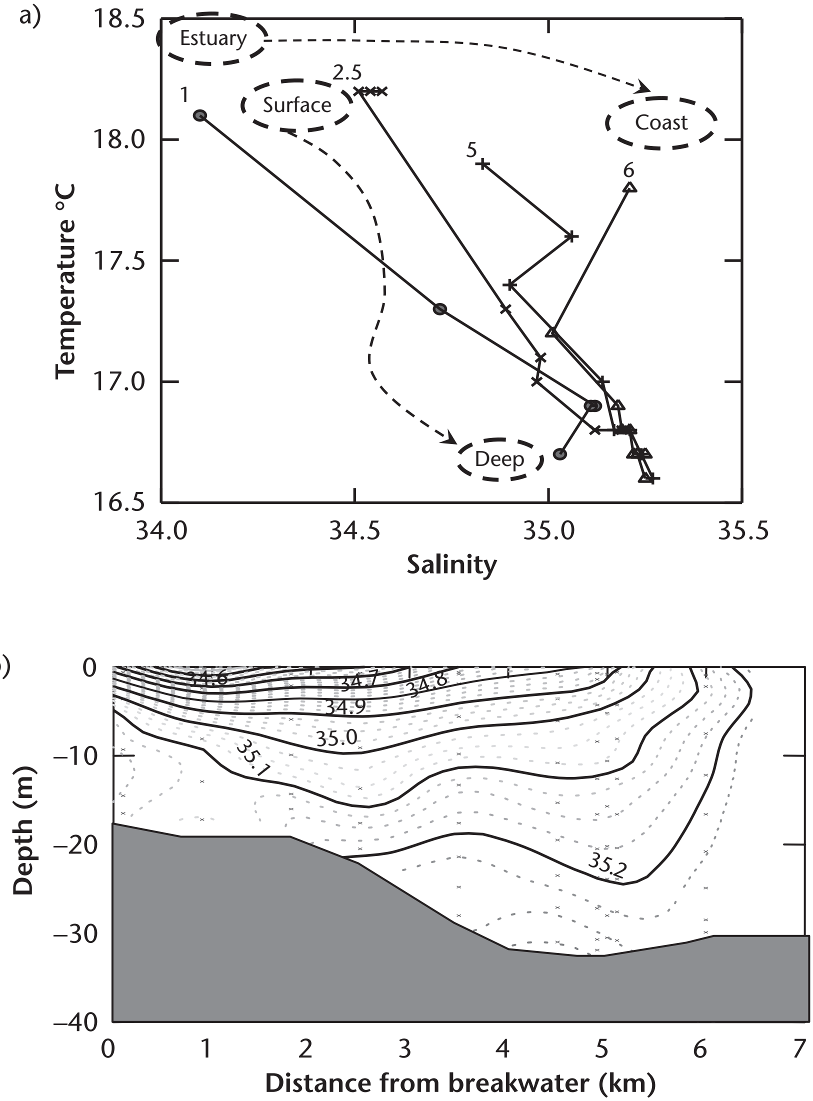


Figure 2.9 a) Example of temperature–salinity (T–S) signatures. The importance of concurrent physical data when collecting plankton is shown in this T–S diagram from within the estuary (1 km from shipping terminal) to the coastal ocean (6 km). At each station, the sampling depth is inferred from least dense (shallow, top left) to most dense (deeper, bottom right). The brackish estuarine plume is evident in the less dense water at stations 1 and 2.5 km. A distinctive estuarine plume front was visible at the surface near Station 5 km (after Kingsford and Suthers 1996). b) Vertical section plot of salinity, from the estuary (left) into the coastal sea (right), showing the surface plume of low salinity water. Arrowed stations are those used in (a) above.

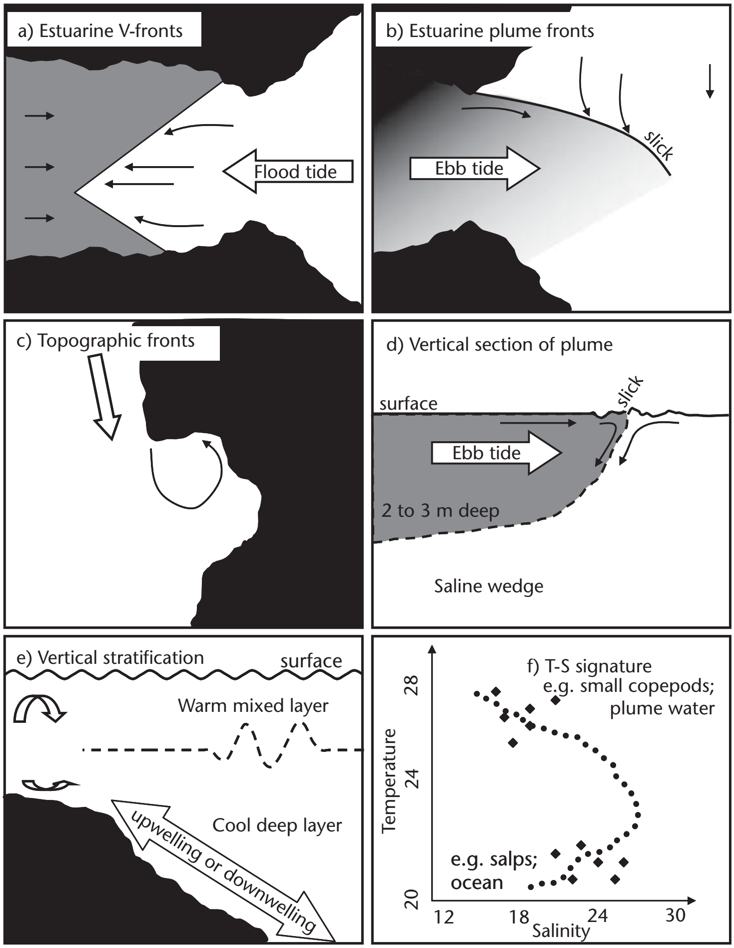


Figure 2.10 Estuarine and coastal habitats: a) A landscape view of an estuarine V-front, as the flood tide is retarded along the channel edge and the saltier (denser) coastal water wedges beneath the estuarine water. b) An estuarine plume front showing the ebb tide flow of brackish (less-dense) water flowing on top of coastal water, which has a coastal flow deflecting the plume. c) A topographic front generated in the lee of a headland or island. d) A vertical section of an estuarine plume front, showing the convergence and sinking along the thermocline or halocline (dashed line) front creating a slick of buoyant material (foam, flotsam). e) Vertical stratification showing a thermocline (dashed line), an internal wave, the breakdown of stratification in shallow water and the potential for upwelling or downwelling. f) T–S signature of a water mass determined from a series of temperature and salinity measurements (line of dots). The depth or distance down-estuary are implied from the least dense (top left) to most dense (bottom right). The dominant types of plankton and water mass associated with particular T–S characteristics are indicated.

may bring deep or benthic plankton near to the surface by eddy pumping (similar to stirring in a tea cup) (Wolanski *et al*. 1996), or by the tidal current scouring around the sides of an island and bringing material to the surface (Suthers *et al*. 2004). Whatever the mechanism, while often complex, the wakes are often obvious from the slightly turbid plumes shown in remote sensing. They can also be seen from aircraft flying above them.

On a calm sunny morning in coastal waters, one may see rows of slicks, 100–200 m apart and parallel to the shore. These are generated by internal waves, which are waves moving along the thermocline (similar to the familiar air–water waves). These waves are created by sudden tide changes or currents at particular submarine cliffs. At the leading edge of each wave is a slight downwelling, which traps any buoyant particles such as oils and, possibly, plankton.

The key to sampling a variable estuarine environment is to always record temperature and salinity with a calibrated electronic meter. Talk to fishers about the local tides and typical currents. Spend some time looking at the waterway with drift objects, such as oranges, to appreciate the individual traits and the appropriate spatial and temporal scales before making any comparisons.

2.7 AN EXAMPLE OF A CLASSIC SALT-WEDGE ESTUARY

The temperature–salinity habitats and the hydrological cycles of tide and seasonal rainfall are the major determinants of estuarine zooplankton ecology. These cycles influence the adaptive responses and behaviours of zooplankton. For example, the Hopkins River estuary is a highly stratified, truncated salt-wedge estuary typical of western Victoria. It is a major river in the region with a catchment area of 8651 km^2 and a mean annual discharge of 295×10^6 m^3. The estuary is only 9.2 km long, and consists of a single, well-defined channel (average width 164 m). The tide is diel (one major low and high per day) with a small semi-diel component. It is normally open to the sea, but closes sporadically due to low rainfall.

Salt-wedge estuaries have a two-layered circulation, with an outflow in the surface layer and net inflow in the bottom layer. As the layer of fresh water moves across the denser salt water of the wedge, turbulent mixing entrains salt water into the upper layer. Water circulation and salinity gradients are the main physical forces that influence the population dynamics of zooplankton (Table 2.1). Mixing processes also affect the productivity of estuaries. Tides or river discharge often introduce nutrients, and wind mixing can re-suspend particulate organic matter along the shallower margins of estuaries. The latter

Table 2.1. Zooplankton assemblages that may occur in estuaries.

Assemblage	Defining characteristics
Marine coastal groups: (a) fully marine (b) coastal marine	Generally these species are strays and are usually non-reproductive Species usually reproduce within the estuary, but predominate in coastal waters
Estuarine groups: (a) estuarine–marine (b) endemic estuarine	Species may extend into coastal waters, but predominate within the estuary Species live and propagate only within the estuary
Freshwater groups: (a) brackish estuarine (b) entirely freshwater	Species extend into the upper estuary Species reproduce in fresh water, but floodwaters can sweep them into the estuary

provides an increased food supply to benthic and planktonic filter feeders, and promotes nutrient exchange between the sediments and the water column.

True estuarine forms dominated the established zooplankton and ichthyoplankton fauna of the Hopkins River estuary. Of significance was the dominance of the calanoid copepod *Gippslandia estuarina* – a situation unparalleled elsewhere. The Hopkins may be an important 'refuge' for primitive or rarer species such as *G. estuarina*. An important link for

BOX 2.3 SAMPLING METHODS IN THE HOPKINS RIVER ESTUARY

Over a 20-month period, a stratified random sampling survey was used to describe the physico-chemical features of the hydrological cycle and the composition and structure of the zooplankton and ichthyoplankton communities. The estuary was divided longitudinally into four main sections and vertically in two layers as separated by the halocline. Section divisions were chosen such that the water chemistry and geomorphology of each section was more homogeneous (similar) than the estuary overall: 1 – moderately deep, incorporates mouth; 2 – relatively shallow, uniform depth; 3 and 4 – presence of deep pools. Sampling sites were chosen randomly (using a gridded map and table of random numbers) within each section for each monthly sampling trip; surface and depth samples were taken at each site. Zooplankton was sampled using a rectangular, perspex, Schindler-type trap with 80 μm mesh outlet – thus enabling more accurate estimation of micro-zooplankton (such as nauplii and rotifers). Ichthyoplankton were sampled using oblique tows with a 250 μm mesh conical plankton net. At each site, surface-to-bottom profiles (at 0.5 m intervals) of salinity, temperature and dissolved oxygen were also measured, as was chlorophyll-*a*, total phosphorus and Secchi depth.

many harpacticoid copepod species, was found between the fauna of the open-water and littoral vegetation habitats within the estuary. In particular, seagrass beds were very important for the copepod *Gladioferens pectinatus* (the second dominant zooplankter of the Hopkins estuary).

The timing of spawning of recreationally important fish species in terms of presence and abundance of ichthyoplankton was found to be linked to the hydrological cycle and the subsequent successional series of the zooplankton (Newton 1996).

Estuarine zooplankton are continuously faced with the risk of being swept out into the ocean, where they may be physiologically stressed or eaten. Zooplankton remain in the estuary by persisting in the layer between the surface brackish water and salt wedge (the halocline) or near the vegetation along the sides and the bottom of the estuary. There is an important link between the limnetic and littoral habitats within the estuary. The Hopkins River estuary generally undergoes annual scouring floods that remove saline waters from the estuary as well as the bulk of the zooplankton community. The persistence of endemic zooplankton populations must therefore be dependent upon effective mechanisms of population re-establishment following the flood phase.

Dormant life history stages appeared to be widespread among the estuarine zooplankton and meiofauna. The presence of dormant eggs among true-estuarine calanoid and harpacticoid copepods was found for the first time (Newton and Mitchell 1999). Other taxa (mainly facultative zooplankters) persisted in the estuary under flood conditions among littoral vegetation, including the calanoid *Gladioferens pectinatus* – a dominant open-water zooplankter of the system. No evidence was found for post-flood inoculation of zooplankters from the marine environment into the estuary.

The strategies used by zooplankton in this study suggest that there is an important adaptive link between estuarine zooplankton and hydrology, and that hydrological cycles are a major structuring force for zooplankton community ecology in salt-wedge estuaries. Furthermore, the successional series, reproductive strategies and behavioural traits of many taxa suggest that the zooplankton community in the Hopkins River estuary is well adapted to the flood disturbance process.

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Chapter 3

Plankton-related environmental and water-quality issues

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3.1 COASTAL WATER DISCOLOURATION AND HARMFUL ALGAL BLOOMS

Phytoplankton are able to reproduce rapidly in favourable conditions. If conditions are suitable, a population explosion – or bloom – can occur (see Figures 6.3, 6.4). Blooms can be red, green, purple, yellow, brown, blue, milky or even colourless. They may be natural or the result of human activities. Some blooms are beneficial to the ecosystem, while others can be harmful, so it is important to know what species make up the bloom and what conditions caused the bloom. Some water discolourations are unrelated to phytoplankton and are a result of silty water (reddish) or drainage from acid sulphate soils (greenish).

Natural phytoplankton blooms in coastal waters may be due to fluctuations in the essential nutrients (such as nitrate, phosphate and silicate), from either an oceanographic upwelling or run-off. Such blooms may be simply harmless transient pulses in response to episodic nutrient enrichment, such

as from coastal upwelling events, when cold, nutrient-rich bottom waters are advected to the surface by winds or ocean currents (see Chapter 2). Sometimes the nutrient enrichment and resultant biomass of phytoplankton is beyond the natural capacity of the environment to assimilate the algal growth (or 'production') – this is known as eutrophication. Eutrophication can affect fish resources, human health and ecosystem function, as well as the recreational amenity of beaches and embayments. Whatever factors affect their formation, the incidence of algal blooms is increasing, as evident in the increased global distribution of paralytic shellfish poisoning (Hallegraeff *et al.* 2003).

Phytoplankton blooms have different effects depending on the types of species that make up the bloom. Some may cause harmless water discolouration; some may be non-toxic, but may be harmful to marine organisms (by either rotting and decreasing oxygen or by shading seagrass); and some may contain potent toxins that are harmful to fish, marine mammals and humans. Phytoplankton blooms that have the potential to cause harm are commonly referred to as harmful algal blooms (HABs).

Most blooms are simply harmless water discolouration (see Figure 6.5). However, if algal blooms are sufficiently extensive (especially in enclosed or partially enclosed areas, such as coastal lagoons and estuaries), it is possible for them to cause fish kills. This may be due to changes in dissolved oxygen availability or by mechanical damage to fish gills. Phytoplankton spines, such as those observed in the diatom genera *Chaeotceros*, may lodge in fish gills and cause an inflammatory response, making them susceptible to infection.

Human illness associated with HABs is due to the naturally occurring toxins that are transferred to humans through the consumption of shellfish or fish. Typically shellfish simply filter toxic phytoplankton and remain unaffected, while the toxins are retained. The most significant public health problems caused by HABs are Amnesic Shellfish Poisoning (ASP, see Box 6.3), Ciguatera Fish Poisoning (CFP), Diarrhetic Shellfish Poisoning (DSP), Neurotoxic Shellfish Poisoning (NSP) and Paralytic Shellfish Poisoning (PSP). Each of these syndromes is the result of different phytoplankton that produces a range of toxins and risks to humans. All these syndromes are caused by toxins synthesised by dinoflagellates except for ASP, which is caused by diatoms (Hallegraeff *et al.* 2003).

Ciguatera Fish Poisoning (CFP) is a severe illness in the short term causing vomiting and diarrhoea, but the long-term effects include tingling in the fingertips, and where hot feels cold, and vice versa, for many years.

It typically occurs when people eat certain fish from near coral reefs, such as some snapper, some mackerel and some surgeon fish. The food chain leading back to the toxic dinoflagellate (*Gamberdiscus*) can be complex – including copepods, shellfish and other prey species – but the affected species of fish are usually known and avoided at certain times of the year.

A relatively recent type of harmful algal bloom is known as 'estuarine associated syndrome'. This is caused by the release of toxic aerosols from two ichthyotoxic dinoflagellates belonging to the genus *Pfiesteria*.

Tasmanian PSP in the Derwent and Huon Rivers is caused by the dinoflagellate *Gymnodinium* (but is also caused by *Gonyaulax*). It was introduced by ballast water in the early 1980s, as determined by their characteristic cysts in layered (that is, dated) sediments. The cysts can remain viable in the mud for many years. *Gymnodinium* typically blooms after a sequence of events: water temperatures higher than 14°C, a rainfall trigger, followed by calm conditions for 14 days (Hallegraeff *et al.* 1995). Once established, wind mixing can prolong the *Gymnodinium* bloom – causing a crisis in the oyster industry.

Potentially toxic phytoplankton are not always toxic in every situation and it is anticipated that other phytoplankton species may prove to be toxic in the future under certain conditions. Only about 40 of the more than 1200 species of dinoflagellates are known to be toxic. Many are very beneficial to the environment and to aquaculture. *Symbiodinium microadriaticum* is important as one of the various symbiotic algal cells ('zooxanthellae') that make up our tropical reef corals – providing coral with essential sugars and beautiful colours.

Shellfish harvesters and aquaculturalists work together with natural resource managers to develop effective HAB management programs. These can include quality assurance programs, biotoxin management programs and algal contingency plans to prevent any harm to the public. Management of blooms requires providing information to the public and waterway users about the causes of blooms and the relevant issues, such as toxicity. Preventing or reducing the discharge of excess nutrients into estuaries and the coastal zone is the most effective means of managing eutrophication. Understanding the pathways of nutrient enrichment taking place in each system is essential.

In urban areas, possible strategies include education programs, source controls, removing pollutants, upgrading sewerage systems, replanting riparian zones and even maintaining good abundances of natural filter

BOX 3.1 INVASIVE SPECIES FROM BALLAST WATER

Shipping movements across the globe have been implicated as the cause of several species of phytoplankton being identified in places where they have not previously been known to occur. Planktonic (and other) taxa are transported in the ballast tanks of ships, having been pumped into the ballast tanks in a port and then pumped out of the tanks once they reach their destination. Harbour environments are an excellent habitat for plankton – often having long residence times and having high nutrient supplies either from the sediment or from the surrounding, generally urbanised, catchments (Hallegraeff 1998).

Ballast water is more likely to transport taxa that are able to survive in conditions where there is no available light, such as dinoflagellates – the survival rates of most photosynthetic plankton would be poor. Once light becomes limiting, such as in a ballast tank, dinoflagellates can form protective coats around their cells (cysts) and sink out of the water column – almost like seeds. Once the ship reaches its destination, the ballast water is pumped out and the dinoflagellate cysts sink to the bottom of the waterway. When nutrients and light become sufficient, the cysts germinate and the resultant cells undergo a reproductive process and the cells begin to grow and multiply.

Studies have identified a large number of species in ballast water. Many of these are cosmopolitan species and do not contain toxins; others, however, contain toxins and have the potential to cause major problems in areas to where they are transported and released. Although ballast water transport makes it most likely that invasive species will be restricted to international shipping ports, secondary transport is possible by smaller local vessels going to smaller ports, such as fishing ports.

Preventing the transport of species in ballast water is difficult and requires global cooperation. Strategies include:

- re-ballasting when there are no obvious algal blooms in ports
- re-ballasting at sea, or flushing ballast water at sea when conditions are suitable
- treating ballast water, either while taking up or discharging ballast water
- screening ships according to the likelihood of them containing targeted pest species. High-risk ships could then be subjected management treatments.

feeders such as mussels and oysters. In many rural areas, land degradation problems and poor land-management practices have contributed to poor water quality. Clearing of vegetation is a major cause of land degradation and poor-quality run-off.

3.2 GEOGRAPHICALLY PERSISTENT ALGAL BLOOMS IN AN ESTUARY

Some estuaries typically have re-occurring blooms in particular areas. For example, the Berowra estuary near Sydney has a continually high biomass of algae in the middle reaches near Calabash Point. Harmful algal blooms also occur intermittently, which result in closure of the Sydney rock oyster aquaculture facilities situated in the downstream reach of the estuary. Closure of the estuary following algal blooms has a significant impact on the local community, due to the importance of the area for boating and swimming. Berowra Estuary is a drowned river valley estuary (tidally dominated), which joins the Hawkesbury River estuary about 24 km from the Pacific Ocean. The estuary has a waterway area of about 13 km^2 and drains a catchment of approximately 310 km^2. A study was instigated to determine when and why the blooms occur in the mid-reaches of the estuary (Rissik *et al.* 2006).

The flushing time in Berowra Estuary was influenced most by the volume of water in each section of the estuary. Flushing time is the time taken for water in a specified region of the estuary to be moved from this region due to replacement (dilution) by incoming fresh water or by tidal dynamics. The volume of water at each reach was determined by the depth and width of the estuary. Upstream the estuary is narrow and fairly shallow; mid-stream the estuary is wide and deep; and downstream the estuary is wide and shallow. These factors translate to flushing times of 1.5 days for the upstream site, 7 days for the midstream site and 1 day for the downstream site (Figure 3.1). Flushing times in the mid-stream reach were sufficiently long for both primary and secondary production to take place in warm summer temperatures.

Primary production was greatest in the mid-reaches (bloom area), indicating that conditions supported rapid growth. Zooplankton was more abundant in the areas with the highest phytoplankton biomass. Small zooplankton was found to respond most rapidly to changes in the phytoplankton. This increase in concentrations of small-sized zooplankton, which were dominated by copepod nauplii, suggested that when more food was available, zooplankton production took place. The high levels of phytoplankton concentrations in the mid-reaches of the estuary indicated that their production was at a rate at which biomass could not be controlled by zooplankton grazing. Only when other factors that reduced primary production rates, such as reduced light intensity, occurred, could the zooplankton assimilate the bloom.

From a manager's perspective, flushing times in various reaches were an important determinant of phytoplankton biomass. To reduce blooms

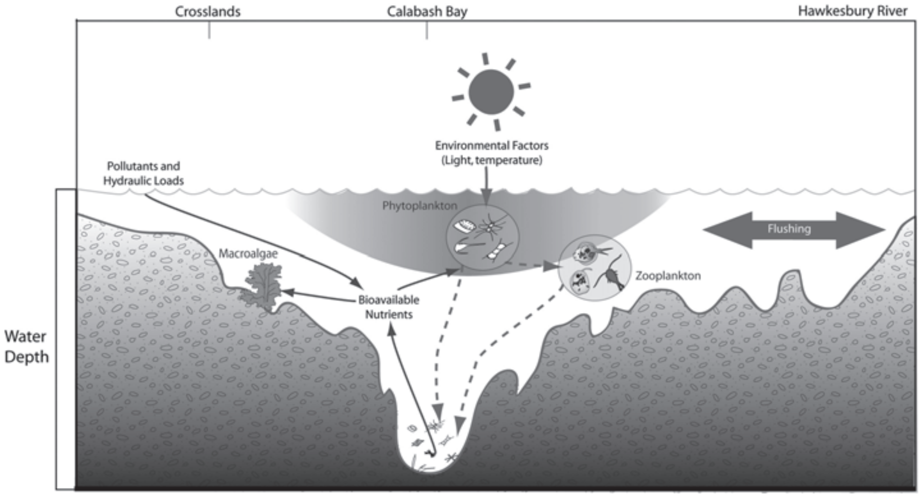


Figure 3.1 A cross-sectional view of the Berowra estuary (northern Sydney, flowing into the Hawkesbury River estuary) showing the relationship of depth (water volume) and thus water retention time, matching doubling time, which results in phytoplankton blooms.

in the mid-reach, flushing times in the deeper sections would have to be reduced to periods of 1–2 days which would involve undertaking works such as filling the deep holes to reduce the depth of the estuary. Such highly engineered solutions would be prohibitively expensive and would have major impacts on the estuary's ecology. Unfortunately, zooplankton grazing was unable to consistently control phytoplankton biomass during warm temperatures and light intensities of summer, as such grazing would only be likely to reduce the biomass effectively if phytoplankton production rates declined.

The most effective options to reduce blooms are those which result in less nutrients being discharged to the estuary from run-off, sewage discharge, directly from homes and some boats. The estuary receives tertiary treated discharge from two sewage treatment plants and also receives stormwater from a number of drains. Solutions were delivered by working with sewage-treatment managers, undertaking educational campaigns, building nutrient-reduction devices, such as constructed wetlands and gross pollution traps, and repairing broken sewerage

infrastructure in the catchment. To assist management, an algal bloom monitoring buoy was moored near Calabash Point, which automatically sends an e-mail to the local council when the chlorophyll-*a* level exceeds 20 $\mu g.L^{-1}$.

3.3 MONITORING PHYTOPLANKTON OVER THE LONG TERM

Red tides have become a common sight in Sydney's coastal waters, often during the spring and summer months. Frequently mistaken for a pollution event (such as dumped paint), blooms of phytoplankton may be highly visible and raise public concern (Figure 3.2, page 129). About 60% of Sydney's reported red tides are formed by surface concentrations of the dinoflagellate *Noctiluca scintillans* (Ajani *et al.* 2001a; Figure 3.2).Fortunately, this species is considered to be non-toxic. In fact, the species is distributed worldwide and is often present in pristine waters. Red tides of *Noctiluca* may cause some irritation to the skin and eyes for those that come into contact with it. Fish and other marine organisms may avoid the bloom area due to the concentrations of ammonia associated with the bloom. Ammonia is produced in vacuoles of *Noctiluca* cells increasing their buoyancy causing increased ammonia concentrations in the water column, especially during the end stages of blooms.

The first detailed study of marine plankton (fortnightly sampling) in Sydney's coastal waters was made in 1931 (Dakin and Colefax 1933). Regular sampling of coastal ocean waters for nutrients and temperature commenced in 1940 offshore from Port Hacking, and continues to the present day, which is the longest record for Australian coastal waters (see literature review in Ajani *et al.* 2001b). Currently there is no coordinated state-wide monitoring program for marine and estuarine plankton for NSW coastal waters. Generally, sampling is limited to bloom events, mariculture and some small-scale monitoring by local councils.

Regional scale oceanographic processes are the main mechanisms for driving seasonal variability of plankton communities for the NSW coast. Increased flow of the East Australian Current (EAC) and upwelling-favourable northerly winds during the spring–summer months stimulates slope water intrusion events that bring cold nutrient-rich water into the coastal zone and encourage phytoplankton growth (Figure 3.2). Oceanographic studies suggest that during peak-EAC flow, back-eddies can form downstream of the area around Forster–Port Stephens, entraining and

incubating phytoplankton as they rotate and displacing them further southward as the eddies move down the coast (Lee *et al.* 2001b).

The waters of the EAC originate in the Coral Sea and are characteristically oligotrophic or nutrient poor. Sydney's deep ocean outfalls are the main continuous anthropogenic source of nutrients to the coastal zone off Sydney, mainly in the form of ammonia and were considered as a potential cause of an apparent increase in visible algal blooms. In comparison, slope-water intrusions deliver episodic influxes of nitrogen (as nitrate) up onto the shelf and towards the coastal zone. Research has shown that blooms appear in response to slope water intrusion events and irrespective of the proximity to other major nutrient sources such as major riverine discharges or Sydney's deep ocean outfalls (Pritchard *et al.* 2003).

Weekly sampling of phytoplankton by Ajani *et al.* (2001b) at the Port Hacking stations concluded that diatom blooms appeared to occur in response to slope-water intrusion events that lasted for a period of 2–22 days during spring and summer. Bottom- and surface-water nutrients and temperature explained 60% of the phytoplankton variability during the study. Additionally, diatom blooms occurred on a similar frequency and magnitude, and in similar species succession patterns, to those found by Hallegraeff and Reid (1986) in 1978–79. Generally, blooms begin with small chain-forming diatoms (*Skeletonema, Thalassiosira, Leptocylindrus, Asterionella*), followed by large diatoms (*Eucampia, Detonula, Lauderia*) and finally by large dinoflagellates (*Protoperidinium, Ceratium*).

Nevertheless, the dominance of the small diatom *Thalassiosira partheneia* (Figure 3.2) and an increased presence of *Noctiluca scintillans* during 1997–98 sampling were unprecedented (Ajani *et al.* 2001b). Factors contributing to the dominance of these species may be related to climate. Comparatively lower concentrations of nutrients and overall warmer water temperatures occurred relative to previous years when eastern Australia was experiencing the effects of an El Niño–Southern Oscillation (ENSO) event (Lee *et al.* 2001a). Warmer water temperatures and strong southward flow of the EAC were also reflected in the increased presence of tropical indicator species (such as *Bacteriastrum, Ceratium gravidum* and *Trichodesmium erythraeum*) compared with three decades ago (Ajani *et al.* 2001b).

Spring and summer blooms of *Noctiluca* at the Port Hacking stations occurred during, or soon after, diatom blooms dominated by *Thalassiosira* and examination of the cell contents of *Noctiluca* confirmed *Thalassiosira* as the dominant prey item (Dela-Cruz *et al.* 2002). Additionally, laboratory studies have found *Thalassiosira* to be an optimal food source for *Noctiluca*.

The shift towards *Thalassiosira* as the dominant diatom bloom species may be the contributing factor towards the increased and year-round prevalence of *Noctiluca* in NSW coastal waters (Ajani *et al.* 2001b).

El Niño is not a recent phenomenon, whereas the year-round presence of *Noctiluca* appears to be unique. While slope-water intrusions are the dominant factor leading to the development of blooms, it is difficult to completely discount nutrients from ocean outfalls as having any effect on phytoplankton trends in NSW coastal waters. Variability in phytoplankton populations due to sewage-derived nutrients may be masked by the larger variability provided by El Niño (Lee *et al.* 2001b). Continuous longer-term data sets are required to distinguish these trends.

In summary, it appears that diatom blooms are not occurring with greater intensity or frequency than in the pre-1980s, although the red-tide forming dinoflagellate, *Noctiluca*, appears more prevalent. Certainly, diatom blooms are natural phenomenon. Long-term monitoring is required to resolve the effects of climatic variability, such as El Niño, on phytoplankton populations compared with increasing anthropogenic nutrient loads and chronic impacts.

Of greater concern is the potential for a shift in prey species. That is, there is the potential for an increase in occurrence of a phytoplankton species that is the preferred food source of a harmful algal species. Blooms of harmful algal species such as *Alexandrium* spp., *Gymnodinium* spp., *Karenia* spp., *Dinophysis* spp. and *Pseudonitzschia* spp. have occurred in south-eastern Australian waters. Toxic algal blooms are a significant potential threat to our coastal environment, local economies and a risk to human health. Modern research methods using remote sensing techniques and on-ground implementation of a state-wide network of moored long-term ocean reference stations would provide an opportunity to monitor physio-chemical and biological oceanography on better spatial and temporal scales.

3.4 PROCESSES UNDERLYING BLOOMS OF FRESHWATER CYANOBACTERIA (BLUE-GREEN ALGAE)

Algal blooms cause a number of problems for managers of fresh water. Surface scums may occur during blooms of blue-green algae (cyanobacteria), flagellated green algae and euglenoids, as these organisms can float or swim to the surface and accumulate. The presence of these scums, and other growths, can lower the aesthetic and recreational amenity of water bodies. Blooms of cyanobacteria impart musty, earthy tastes and

BOX 3.2 EFFECTS OF EUTROPHICATION

Eutrophication is the process that increases biological productivity within an ecosystem and in particular algal blooms. The causes are many, but are usually associated with an increase in nutrients from agricultural or sewage run-off. Algal blooms can cause large daily variations in pH and dissolved oxygen. By day, algal photosynthesis removes carbon dioxide from the water, allowing the pH to increase, and produces oxygen, which can lead to supersaturation of dissolved oxygen. At night, cellular respiration by the algae, and other organisms in the water, increases the amount of carbon dioxide dissolved in the water, and causes pH to fall, while dissolved oxygen can fall to quite low concentrations. Large daily changes in pH in raw waters used for town water supply are not desirable, as water-treatment processes work best at a constant pH. Low dissolved oxygen concentrations at night may place stress on fish and other aquatic organisms. Decomposing cells and the absence of oxygen can also lead to the production of noxious gases, such as hydrogen sulphide and methane, and to high concentrations of ammonia, which may be toxic to aquatic organisms. Anoxic conditions also lead to reducing chemical conditions at the sediment–water interface, and the mobilisation of soluble forms of nutrients, especially phosphorus, from the sediments, which can lead to future algal blooms. Metals – in particular iron and manganese – are also mobilised under anoxic conditions, and their presence in a town water supply can cause discolouration, taste and staining of laundry.

odours to the water, while blooms of green algae can impart grassy tastes and odours, and blooms of some chrysophytes and other flagellated algae can create fishy tastes and odours. The presence of numerous algal cells in the water can also cause problems for water treatment plants by blocking filters and other water-treatment equipment, and fine nozzles in irrigation systems.

There are a number of environmental factors that drive the formation of algal blooms in freshwater environments. Although often considered individually, it is often the coincidence of several factors operating together that may lead to a bloom. In addition, because of the wide diversity in freshwater algae, different species have considerably differing environmental tolerances and requirements, so that one set of water-quality characteristics may suit some species of phytoplankton, while another set may suit completely different species. For example, blooms of cyanobacteria may be enhanced by nutrient-enriched, warm waters that are slightly alkaline, while chrysophytes may predominate in cold, soft, oligotrophic waters that are slightly acidic. This section will concentrate on the factors

causing cyanobacterial (blue-green algal) blooms in fresh water because of their relative importance in terms of public health and hazard and risk to livestock and wildlife, and to their frequency in comparison to blooms of other types of algae.

3.4.1 Nutrients and other limiting factors

Cyanobacterial blooms are driven by an increased presence of nutrients. The nutrient in fresh water that is usually attributed to causing most algal blooms – and cyanobacterial blooms in particular – is phosphorus (Box 3.3). The second major nutrient required by freshwater phytoplankton is nitrogen (Box 3.4). Cyanobacteria and eukaryotic algae also require other micronutrients for growth, such as iron, but these are generally available in concentrations that do not limit growth in most fresh waters. Many temperate latitude species of cyanobacteria that form noxious blooms have optimal growth rates above 20°C (Robarts and Zohary 1987), which occur during spring, summer and autumn.

BOX 3.3 KEY NUTRIENT: PHOSPHORUS

Phosphorus can be measured in two ways – as soluble reactive phosphorus or as total phosphorus. Soluble reactive phosphorus represents the phosphorus that is immediately available for algal growth within the water column. Total phosphorus includes not only the soluble forms, but also that bound up in the cells of existing phytoplankton and other microscopic aquatic organisms, in organic detritus, and in part of the suspended particulate mineral material. Much of the total phosphorus is thus not immediately available for phytoplankton growth, but may become available in the near future. In many Australian inland waters, soluble reactive phosphorus represents only 10 to 30% of the total phosphorus. Although cyanobacteria can grow at lower concentrations, they tend to become more prevalent as total phosphorus concentrations rise, especially above 10 μg L^{-1}. Various algal and cyanobacterial species respond to different total phosphorus concentrations. For example, very tiny celled cyanobacteria from the Order Chroococcales are better able to scavenge available phosphorus at low concentrations than some of the larger celled species, such as *Anabaena circinalis*, which require higher concentrations. In terms of the number of cells present per millilitre of water, the Chroococcales may bloom at low total phosphorus concentrations, although, because of their tiny size, these large cell numbers still represent very little biomass. However, total phosphorus concentrations above 20 μg L^{-1} – and especially above 30 μg L^{-1} – favour most cyanobacteria.

BOX 3.4 KEY NUTRIENT: NITROGEN

Nitrogen availability can be measured in terms of readily bioavailable forms, such as oxidised nitrogen (nitrate and nitrite) and ammonia, and also as total nitrogen, which includes the organic and bound forms of nitrogen as well. Algal presence increases as nitrogen becomes more readily available at higher concentrations, especially once total nitrogen exceeds 1000 μg L^{-1}, provided other factors are not limiting the growth. The form of nitrogen may also influence the type of phytoplankton present. Cyanobacteria from the Order Chroococcales prefer nitrogen to be present in the form of ammonia, while other cyanobacteria and eukaryotic algae more readily use nitrate. Some heterotrophic flagellated algae (such as non-photosynthetic dinoflagellates) may use organic sources of nitrogen. Some cyanobacteria are, however, less reliant on ambient nitrogen concentrations, because they can fix atmospheric nitrogen to obtain their needs if concentrations in the water are low. Nitrogen fixation is especially common in the Order Nostacales, although species from other orders can also do this. Most phytoplankton (diatoms, dinoflagellates), including many cyanobacteria, cannot however fix atmospheric nitrogen.

Cyanobacteria generally prefer calm, non-turbulent conditions within the water column, as this allows them to maximise their buoyancy regulation mechanisms and float towards the surface and light, or to sink into deeper waters as required. Deeper lakes, weir pools and reaches of rivers become thermally layered (stratified) in summer, when their surface waters are warmed up by the sun. This stratification of the water column creates considerable stability and reduces turbulence. Such conditions are ideal for cyanobacterial blooms, but are unsuitable environments for many of the larger, heavier non-flagellated eukaryotic algae, such as green algae and diatoms, which require turbulence to keep them suspended within the water column and to prevent them from sinking.

Algal bloom development is also facilitated by water retention times. Retention times (the period of time required for all the water in a lake, reservoir or weir pool to be replaced by new water) longer than 2 weeks tend to favour cyanobacterial growth (Mitrovic *et al.* 2003). High flow rates in rivers are not conducive for any algal bloom, as the algal cells are displaced downstream (although certain algal species are distinctively riverine and continue to live in discrete packages of water as these move downstream).

The availability of light is another factor that may promote blooms during spring and summer. Phytoplankton cells also need to be close enough to the surface (the euphotic zone) to obtain sufficient light for photosynthesis, so that food production equals, or exceeds, loss by respiration. The maximum depth for photosynthesis is usually considered to be the depth at which only 1% of the light penetrating the surface of the water remains. Light penetration is limited by dissolved organic substances, which often stain the water a yellow to brown colouration, and suspended particulate matter. These substances in the water also change the spectral distribution of the light away from the blue wavelengths that are most useful to algae, towards a predominance of yellow to red wavelengths. This is outside the main range of wavelengths absorbed by chlorophylls, but many algae have additional pigments, such as carotenes and xanthophylls – and in cyanobacteria phycocyanin and phycoerythrins – so that they are still able to harvest light within these wavelengths.

Turbidity or suspended particulate matter is a major factor influencing the underwater light availability of many inland waters. Turbidity is actually a measure of amount of light scattered by these particles, but often used as a surrogate measure of the amount of suspended particulate matter. Cyanobacteria appear well adapted to high and low turbidity. Blooms occur in low turbidity water, where light is plentiful for photosynthesis, and in some weir pools it has been demonstrated that once turbidity falls below a certain level and the water becomes clearer, then the chance of cyanobacterial blooms increases considerably (Mitrovic *et al.* 2003). Blooms also occur in highly turbid water. As well as having ancillary pigments for light harvesting in light-restricted waters, cyanobacteria can use their positive buoyancy in non-turbulent turbid waters to rise to the surface to where there is sufficient light for their needs. Cyanobacteria also have quite low light requirements in comparison with many eukaryotic algae, enabling them to grow in such light-restricted environments and, in fact, prolonged exposure to high light intensities is detrimental – resulting in the death of cells.

Salinity, and the ionic composition of these salts, and pH are additional environmental factors that may have some effect on algal presence in fresh waters. Little is known of the salinity tolerances of most freshwater species of phytoplankton. Two potentially toxic species of cyanobacteria, *Anabaena circinalis* and *Microcystis aeruginosa*, have been shown to have salt tolerances of up to 5 to 6 grams of salt per litre (about 15% seawater) before they are killed off by salinity (Winder and Cheng 1995), which is well above the salinity of water considered to be 'fresh' (about

5% seawater or <3 $g.L^{-1}$). Therefore salinity may select for a particular species of cyanobacterium. For example, changes in species composition from *Anabaena* sp. to the more salt-tolerant *Anabaenopsis* sp. have been indicated in some parts of the Darling River in New South Wales where saline groundwater inflows occur under low flow conditions. In South Australia, *Anabaena circinalis* in the Murray River tends to be replaced by the brackish water species, *Nodularia spumigena*, in Lake Alexandrina, where salinities are higher. The pH tolerance varies from species to species. For example, many chrysophyte algae prefer slightly acidic, soft water environments, while cyanobacteria in general grow better in slightly alkaline waters (8.0–8.5). Blooms of phytoplankton often cause the pH to vary anyway, as they use and replace the carbon dioxide in the water through photosynthesis and respiration on a daily basis.

The main concern about algal blooms is the ability of some, but not all, to produce potent toxins that create a public health hazard and can lead to the deaths of domestic animals and wildlife. In fresh waters, only some species of cyanobacteria are known to produce toxins, although all produce contact irritants. Cyanobacterial contact irritants cause skin and eye irritations and digestive tract upsets in recreational water users who come into contact with them, or swallow water containing them. The potency of these contact irritants varies from species to species, while the response of people coming into contact with them also varies greatly, with some people being particularly susceptible to them, while others are not.

There are two main types of toxins produced by cyanobacteria – those generally termed hepatotoxins and those known as neurotoxins. Hepatotoxins cause the breakdown of the cells within the liver, and other internal organs of the poisoned victim, and may lead to death by internal haemorrhage. Neurotoxins attack the nervous system of the poisoned victim, and may lead to death from respiratory failure. In addition, some of these substances have been identified as cancer-promoting substances. Each year in Australia, cyanobacterial blooms cause the deaths of agricultural livestock drinking from contaminated water sources. The deaths of humans at a renal dialysis clinic in Brazil have also been attributed to cyanobacterial toxins in the water used in their treatment. To date, only seven or eight species of cyanobacteria have been shown to produce these toxins in Australia. Research has indicated that approximately 40% of blooms within the Murray–Darling Basin are toxic (Baker and Humpage 1994), with neurotoxic *Anabaena circinalis* predominating. Hepatotoxic species include *Microcystis aeruginosa, Nodularia spumigena*, and *Cylindrospermopsis raciborskii*. (See Box 3.5.)

BOX 3.5 ANALYSIS OF CYANOBACTERIAL TOXINS

There are a range of methods by which the toxicity of cyanobacterial blooms can be assessed.

Mouse bioassay

This has been the traditional method of toxicity assessment. Concentrated samples of cyanobacteria are required. Known concentrations of sterile cyanobacterial cellular extracts are administered to test mice by intra-peritoneal injection. From these tests, the concentration that will kill 50% of mice (the LD_{50}) can be calculated. The time to death indicates whether the sample is hepatotoxic or neurotoxic–the latter being most rapid. Autopsy also indicates any internal organ damage due to hepatotoxins. Because of animal ethics considerations, mouse bioassays are less frequently used these days.

High-pressure liquid chromatography (HPLC)

This is used to determine the concentration of common hepatotoxins in water samples. HPLC can also be used for the determination of saxitoxin (a neurotoxin) concentrations in water, although different analytical and detection methods are required. There is no one HPLC analysis that will test for all toxins simultaneously.

Liquid chromatography-mass spectrometry (LC-MS)

Also used for hepatotoxin analysis, especially for the toxins produced by *Cylindrospermopsis raciborskii*.

Enzyme linked immunosorbent assay (ELISA)

These employ antibodies raised to react with certain hepatotoxins. Differences in the cross-reactivities of the antibodies used in different ELISA test kits to the range of hepatotoxins possible in environmental samples may influence their relative performance, and produce over or underestimates of toxin concentration. They therefore cannot be relied on as quantitative assays, unless the bloom is ongoing with a known and consistent toxin profile.

Protein phosphatase inhibition assays (PPI)

The hepatotoxin microcystin is a potent inhibitor of protein phosphatases, and a colorimetric test is used to detect this enzyme inhibition. The test can provide overestimations of toxin content as cyanobacterial cellular compounds other than the toxins may also cause inhibition.

Box 3.5 (*Cont.*)

Polymerase chain reaction (PCR)
This method amplifies the DNA within cyanobacterial cells, and detects the presence of gene sequences that code for toxin biosynthesis. As such, it provides a rapid screening test of the potential for the cyanobacteria within a bloom to produce toxins (if the genes responsible for toxin production are present, the bloom can produce toxins – if the genes are absent, the bloom will not be toxic). The test does not provide a quantitative measurement of any toxins present. PCR is currently used mainly as a research tool, and is not yet commercially available for routine sample analysis.

3.5 PHYTOPLANKTON MONITORING IN NEW ZEALAND FOR TOXIC SHELLFISH POISONING

Shellfish are an important resource in New Zealand and have great cultural importance for Maori and, more recently, for New Zealanders of European descent. Over the last three decades, shellfish, particularly Greenshell™ mussels, have formed the basis of a large aquaculture industry (with an annual revenue of more than $200M). Mussels, oysters and other important bivalves are filter feeders of phytoplankton (see Box 3.6) and thus can be a very efficient vector for transferring biotoxins from phytoplankton to humans via the consumption of shellfish. While these naturally occurring toxins are not harmful to the shellfish, they can be fatal to humans. Several large-scale monitoring programs are in place in New Zealand to minimise these threats.

Prior to 1992, Toxic Shellfish Poisonings (TSP) resulting from the consumption of filter-feeding shellfish grazing on phytoplankton had not been officially reported in New Zealand. However, awareness of the risk of toxic phytoplankton was raised in the summer of 1992–93 when 180 cases of illnesses fitting the case definition for Neurotoxic Shellfish Poisoning were reported. Although this event was relatively localised to a section of the North Island coastline, a blanket closure of commercial and recreational shellfish harvesting was enforced nationwide. This seemingly extreme response enabled management structures to be developed, and provided a coordinated approach to contend with the TSP event and future Harmful Algal Bloom (HABs) events.

In this context, New Zealand's National Marine Biotoxin Management Plan (NMBMP) was established. An independent phytoplankton laboratory constitutes the first tier of monitoring for toxic microalgae, which is divided

BOX 3.6 DEPLETION OF PHYTOPLANKTON AROUND NEW ZEALAND MUSSEL FARMS

Mussels are New Zealand's second most valuable export seafood species after hoki. At present there are three primary growing areas in New Zealand: Marlborough Sounds, Firth of Thames and Stewart Island although new coastal areas – and possibly even large offshore blocks – are presently being opened up for farming.

Shellfish growers are farmers: they sow the seed, tend the crop and then harvest the product. Hence, there are many similarities between shellfish aquaculture and horticulture, but there are major differences. Most terrestrial farmers have property rights in the form of land tenure or leases and hence they have control over the land and soil. Terrestrial farmers have the ability to manipulate, in part, the growing conditions through the use of irrigation and fertilisers. By contrast, shellfish farming involves placing the crop in the water and allowing it to grow under the influence of a natural food supply. The farmers have little control over the food availability – food in the form of phytoplankton, zooplankton and detritus simply passes through the farms. Therefore, farmers share food resources and, importantly, the same suspended particles are food for other parts of the marine ecosystem. Therefore, shellfish farmers must live more in the context of naturally occurring processes and have little ability to influence food supply to individual farms or growing areas.

The shellfish industry in New Zealand is relatively young and is still expanding into new growing areas. How many shellfish farms can be established without having an undue adverse effect on the environment (ecological carrying capacity)? Shellfish farming applicants must at a minimum provide predictions of the likely extraction of phytoplankton that will result if a farm is established, and some guide to the possible impacts of this extraction to the greater ecosystem. Predictions are derived from simple analytical models to complex coupled hydrodynamic-ecosystem models. However, the level of uncertainty often increases with the complexity of the models. These models are generally nutrient–phytoplankton–mussel growth models that typically ignore all other plants and animals in the system. The other principal weakness of these types of models is that bottom-up drivers of phytoplankton production are nutrient inputs. In inshore areas nutrients are derived from run-off and, in some cases, from local oceanographic events.

The development of the shellfish aquaculture industry in New Zealand has also led to a renewed interest in the abundance and distribution of phytoplankton in coastal waters. In particular, farmers have an interest in understanding the availability of phytoplankton for farm planning and management – and other stakeholders and regulators have an interest in understanding how the establishment of shellfish farms may influence other marine animals and communities that rely on phytoplankton.

into the commercial (industry) and non-commercial (public health) sectors. The laboratory is accredited to ISO17025 standard and uses the National Reference Collection of Microalgae (maintained at Cawthron Institute). This gives an early warning of potential blooms at up to 250 representational sites around the coast of New Zealand. Risks associated with toxic species are defined by the New Zealand Food Safety Authority and a conservative approach is taken to trigger flesh testing, with regulatory decisions being made based on flesh test results. This introduces the second tier of HAB monitoring – biotoxin testing. In conjunction with water sampling sites, shellfish are collected on a weekly basis and tested for marine biotoxins. If potentially toxic phytoplankton are identified in the water samples, a search for the toxin group is made in the flesh sample. These two complimentary monitoring systems optimise sampling effort, cost and reporting time constraints. For example, where phytoplankton testing represents a spot sample in time, flesh testing resolves these spatial and temporal issues to a degree, because shellfish act as bioaccumulators, concentrating toxins in their flesh. Conversely, a lag period is often observed between the detection of toxic phytoplankton in the water column and when shellfish accumulate the toxin to a level where it is detectable. This lead time provides early warning to managers if further action is required. Therefore, combining phytoplankton and biotoxin monitoring provides a comprehensive, efficient and cost-effective system for detecting HABs and their biotoxins.

For example, the system was used to identify a particular species of *Pseudo-nitzschia* that produces a novel form of domoic acid (iso-DA). Not all species of *Pseudo-nitzschia* produce toxins, but differentiating *Pseudo-nitzschia* species with light microscopy is almost impossible. As a solution to this problem, a suite of DNA probes were developed and are offered as a routine test with compliance to ISO 17025 standard. At one stage, *Pseudo-nitzschia* cells (3.6×10^4 L^{-1}) were present at the same site and time in the Marlborough Sounds as shellfish were found containing iso-DA. Because the phytoplankton monitoring requires both live and preserved water samples, *Pseudo-nitzschia* species from the Marlborough Sounds sites where iso-DA was detected were able to be isolated and cultured from the live water sample. Cultures of each isolate were identified to the species level using DNA probes and stressed to enhance DA production. Analysis of the different forms was carried out using liquid chromatography mass spectrometry (LC-MS) and *Pseudo-nitzschia australis* was identified as the producer of the novel iso-DA.

A bloom of *Gymnodinium catenatum* was tracked as it extended along the coastline of the North Island using phytoplankton and biotoxin monitoring. Low levels of PSP toxins were detected in routine flesh samples off the West

Coast of the North Island and reactive sampling of the water around these areas resulted in the detection of *G. catenatum.* Routine sampling for phytoplankton monitoring was limited in this area by high surf and the exposed nature of the coastline. From the original point of detection, it soon became clear that the bloom was intensifying and expanding – both in terms of cell numbers and shellfish toxicity levels. Within one month, *G. catenatum* had spread into Ninety Mile Beach (far north of the North Island), with resting cysts of this species detected in high numbers. Resting cysts can germinate later into the usual form of the species when environmental conditions are favourable – sometimes many years later. This posed a major problem to the industry as contaminated drift weed that naturally washes ashore on this beach supplies around 80% of mussel spat required for seeding out mussel farms around New Zealand. With the detection of *G. catenatum*, a voluntary ban was imposed to prevent transport of contaminated weed to unaffected areas around New Zealand. The future production of the mussel industry was in serious jeopardy as it faced spat shortages for their next seasons' crop. Compounding this problem was the timing of the bloom, which coincided with the prime collecting time for spat and for re-seeding marine farms. In response to the dilemma marine farmers and the industry were facing, several methods were developed to eradicate cysts from the weed to which the spat were attached. Decontamination of spat at cleansing plants allows 'clean' spat to be transferred into unaffected aquacultural areas, such as the Marlborough Sounds.

Although this was the first recorded presence of this species, sediment cores taken from around highly affected areas suggest that resting cysts have been dormant in the sediments since at least 1981, and even as far back as 1921 in some areas. This inferred that *G. catenatum* was not a recently introduced species, as first speculated, but had in fact been in New Zealand waters in recent history. There will always be new species discovered, new toxins detected, new regulatory demands and the need for new technologies to be developed. The monitoring program must be adaptive and amenable to evolve at this rate to best mediate the effects of HABs and marine biotoxins.

3.6 FRESHWATER ZOOPLANKTON AS INTEGRATORS AND INDICATORS OF WATER QUALITY

Monitoring and assessment of the freshwater environment are often based on turbidity, pH, dissolved oxygen, biological oxygen demand and nutrients. Point measurements of these physio-chemical traits can vary over hours to weeks, and from metres to kilometres, whereas we need traits that

integrate the small scale variation. Zooplankton have been used widely as indicators to monitor and assess various forms of pollution including acidification, eutrophication, pesticide pollution and algal toxins. In addition, zooplankton have been used to improve water quality, particularly using the knowledge of their feeding behaviours. Examples of biomanipulation and mosquito control are presented below.

In the northern hemisphere, acidification (that is, the lowering of pH) due to acid rain, resulting from airborne pollutants such as sulfur dioxide and nitrous oxides, has had adverse effects on a broad range of organisms in freshwater ecosystems. Zooplankton species richness is reduced with increasing acidification. The cladoceran or water flea, *Daphnia*, is eliminated, while smaller crustaceans (especially *Bosmina* and some calanoid copepods) and rotifers become dominant. With the concomitant loss of fish, cyclopoid copepods may become the top predators in the lake, together with macroinvertebrates such as corixid bugs and phantom-midge larvae.

The relative abundance of the rotifer *Keratella taurocephala* is a good indicator of low pH in North American lakes, while the littoral cladocerans *Alona rustica* and *Acantholeberis curvirostris* are associated with acidic lakes in Norway. Zooplankton have been used to assess natural and artificial recoveries of lakes from acidification by the addition of lime. With recovery of acidified lakes, the increase in species richness and return of acid-sensitive species of zooplankton have been reported (Keller *et al.* 1992; Locke and Sprules 1994; Walseng and Karlsen 2001).

Eutrophication of lakes and ponds also changes the size structure, species composition, and biomass of zooplankton. Typically, total zooplankton biomass increases with increasing eutrophication and is accompanied both by species and groups replacement, and increased importance of rotifers and ciliated protozoans. Cyclopoid copepods and cladocerans assume greater importance relative to calanoid copepods with eutrophication, and large cladocerans are replaced by smaller taxa in eutrophic lakes. Some of the zooplankton species are specific indicators of either eutrophy or oligotrophy in temperate lakes in the northern hemisphere (Table 3.1). The rotifer *Asplanchna brightwelli* is listed as an indicator of eutrophy in an Australian river (Shiel *et al.* 1982).

In addition, the process of lake eutrophication in the past can be studied by means of the examination of exoskeletons (exuviae) of cladocerans in sediments. By checking abundances and changes in species compositions of the cladoceran remains collected in sediment core samples, the timing and trajectory of eutrophication and loss of littoral habitats are inferred and used to support other paleolimnological evidence of lake eutrophication (Jeppesen *et al.* 2001).

Table 3.1. Indicators of trophic status in lakes in the northern hemisphere (Gannon and Stemberger 1978; Gulati 1983).

Trophic status	Animal group	Species
Eutrophy	Rotifers	*Anuraeopsis fissa, Brachionus angularis, Filinia longiseta, Keratella cochlearis* f. *tecta, Polyarthra euryptera, Pompholyx sulcata, Trichocerca cylindrica* and *Trichocerca pusilla*
Oligotrophy	Calanoid copepods	*Limnocalanus macrurus* and *Senecella calanoides*

Discharge of pesticides, such as herbicides and insecticides, from agricultural and pastoral lands into rivers and dams has adverse effects on the freshwater environment and human health. Zooplankton have been used as test or monitoring organisms to assess the acute and chronic toxicity, bioconcentration and biomagnification of these chemicals. In normal agricultural practice, protection of crops from pest organisms is achieved with the application of more than one chemical for different target organisms. The effects of combinations of pesticides on freshwater ecosystems may be synergetic, resulting in greater harm than expected.

Large cladocerans and calanoid copepods in general are more sensitive to pesticide toxicity than microzooplankton, such as *Bosmina, Ceriodaphnia*, rotifers and cyclopoid copepods. Therefore, an increase in microzooplankton could occur following pesticide applications, which may lead to an increase in certain groups of phytoplankton due to decreased zooplankton grazing pressure (Hanazato 2001). The feeding performance of zooplankton such as *Daphnia* is inhibited by sublethal concentrations of the pesticide endosulfan (DeLorenzo *et al.* 2002).

The cyanobacteria *Microcystis* and *Anabaena* may produce intracellular toxins and release them into surrounding waters, especially when they are in a senescent growth phase or when an algicide has been applied. Zooplankton such as *Daphnia*, copepods and rotifers are used ecotoxicologically as test organisms to assess the direct and indirect effects of cyanotoxins. High concentrations of cyanotoxins kill zooplankton, including *Daphnia*, while low concentrations of cyanotoxins reduce the growth and reproduction of various zooplankton (DeMott *et al.* 1991; Gilbert 1994). Even filtered water that had been used to grow toxic cyanobacteria (such as *Anabaena*) is reported to have a negative effect on *Daphnia*'s feeding activities (Forsyth *et al.* 1992). Warmer temperatures may exacerbate the effects of cyanotoxins on zooplankton. Zooplankton such as *Daphnia* can

accumulate cyanotoxins in their bodies and may transfer them to higher trophic levels, such as fish.

3.6.1 Remediation of phytoplankton blooms and biomanipulation

Phytoplankton often increase excessively in eutrophic water bodies, causing reduced water transparency, the production of toxins, a foul odour and clogging of filters in water treatment facilities (see Section 3.4). One way to control excessive phytoplankton abundance is to reduce the amount of nutrients entering the water. Phosphorus is one such nutrient and is present in many detergents that can end up in waterways. This is why people are urged to use phosphorus-free detergents at home. Another way is to encourage herbivorous zooplankton, particularly *Daphnia*. In lakes, for example, phytoplankton are eaten by zooplankton, and zooplankton are eaten by fish. The removal or reduction of zooplanktivorous fish stimulates the growth of zooplankton, which will then eat more phytoplankton. Reduced phytoplankton abundance will lead to an improvement of water quality and clearer water.

Biomanipulation is the term applied to such manipulations of the biota and of their habitats to facilitate biological interactions that result in the reduction of excessive algal biomass – in particular, of cyanobacteria (Shapiro 1990; Carpenter and Kitchell 1992). The biomanipulation approach includes the introduction of phytoplankton-eating fish and control of macrophytes (large plants). It focuses on the manipulation of zooplankton-eating fish and zooplankton to increase grazing pressure on phytoplankton. Biomanipulation has been used in ponds, lakes and reservoirs, particularly in the northern hemisphere. Because biological interactions are often very complex in aquatic ecosystems, the biomanipulation trials can meet with both success and failure. The average success rate of biomanipulations is reported to be about 60% (Mehner *et al.* 2002). Biomanipulation is most likely to be successful in shallow eutrophic lakes.

3.6.2 Mosquito control

Studies have been carried out on the use of carnivorous copepods (especially the cyclopoids belonging to the genus *Mesocyclops*) as biological agents for control of mosquito larvae in wells, mines and other breeding habitats, especially where mosquito-eating fish are not effective in controlling them (see, for example, Russell *et al.* 1996). Such studies are important, as certain mosquitoes are a vector of viruses that cause fatal diseases to humans (such as Dengue and Ross River fevers). Carnivorous copepods

may be used as an environmentally acceptable and persistent agent for the control of such mosquitoes if operationally feasible procedures for the rearing and field introduction of carnivorous copepods are established.

3.7 GRAZING AND ASSIMILATION OF PHYTOPLANKTON BLOOMS

The assimilation of eutrophication is an under-appreciated management consideration for maintaining water quality. The invasion of the Great Lakes in the north-eastern US by the zebra mussel (*Dreissena polymorpha*) has fundamentally altered the ecology of those lakes. By filtering out the lakes' phytoplankton, zooplankton populations have collapsed and so have the zooplanktivorous fish (such as the 'alewife' *Alosa pseudoharengus*, which was also introduced). Zebra mussels are also found to decimate the phytoplankton concentration in Hudson River and San Francisco Bay. Recently, the pygmy mussel *Xenostrobus securis* has been implicated in the rapid demise of phytoplankton blooms in the Wallamba River (central coast of New South Wales, Moore *et al.* 2006). *Xenostrobus* aggregates on the mangrove aerial roots in brackish waters. Up to 25% of the decline in phytoplankton blooms was attributed to the pygmy mussel, but the remaining 75% (unrelated to hydrography) could be caused by zooplankton or population decay by salinity stress (Moore *et al.* 2006).

Zooplankton can reduce the frequency of harmful algal blooms by keeping bloom species at low concentrations via grazing (Chan *et al.*, 2006), and the zooplankton biomass can increase. Analysing sufficient zooplankton samples to understand the interactions taking place in estuaries can be time consuming and answers can be achieved more rapidly by using a particle counting and sizing device. The abundance of various size categories of zooplankton can yield a useful estimate of grazing and production rates, because metabolic rate is predictably related to body size (Section 2.1). Biomass is passed from smaller to larger particles via predation (Figure 3.3). Particle size is measured by an optical plankton counter or image analysis as area, which is converted to biomass assuming a density of water and the volume of a sphere (see Section 4.9). The slope of the NBSS is theoretically around –1 (Figure 3.3), which serves as an index of zooplankton production, although the interpretation is complicated by both top-down (predation) and bottom-up (nutrient) effects.

To assess the effect of catchments on zooplankton, we determined the size frequency distribution of zooplankton in three contrasting NSW estuaries using an optical plankton counter (Moore and Suthers 2006). One

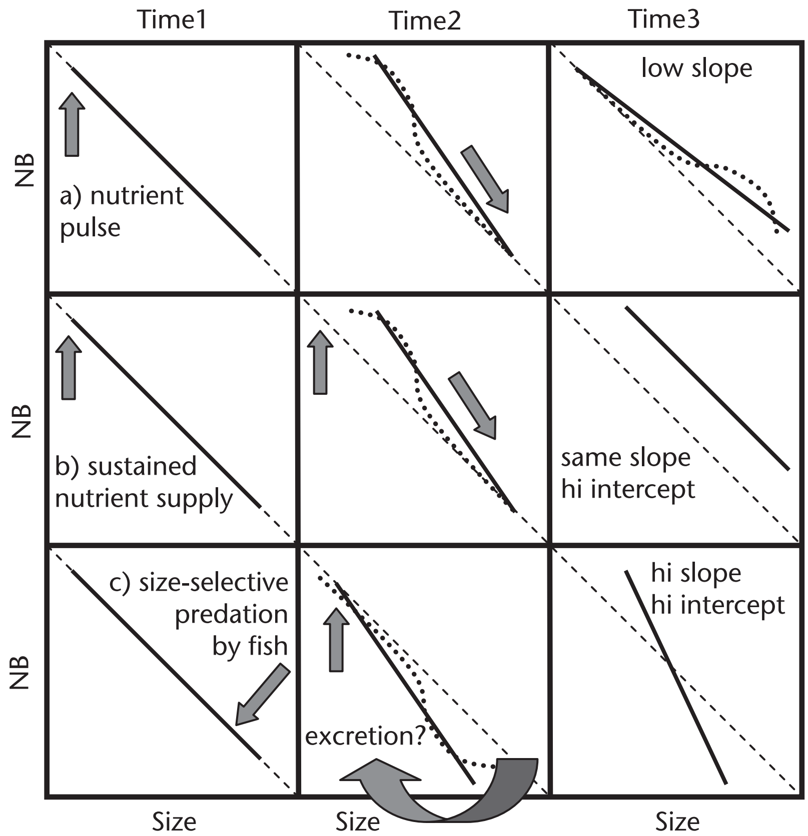


Figure 3.3 Sketch of possible bottom-up and top-down processes altering the –1 slope and intercept of the zooplankton NBSS (Normalised Biomass Size Spectrum) around Cato Reef, during three time periods. a) A nutrient pulse stimulates phytoplankton and increasing the (normalised) biomass concentration of small zooplankton particles, which is passed by predation to larger particles. b) A sustained nutrient supply increases the biomass and intercept. c) Size-selective predation by larval and juvenile fish could steepen the slope, and their excreted nutrients could increase the production of smaller particles (adapted from Suthers *et al.* 2006).

estuary had a forested and less-developed catchment (the Wallingat River) while the other two estuaries had catchments dominated by dairy farming and hence had enhanced nutrient flows. Zooplankton was collected by towing a 100 μm mesh net at replicated stations. We found the monthly variation was related to rainfall and nutrient supply to the estuaries. There were significant differences in the zooplankton NBSS between large

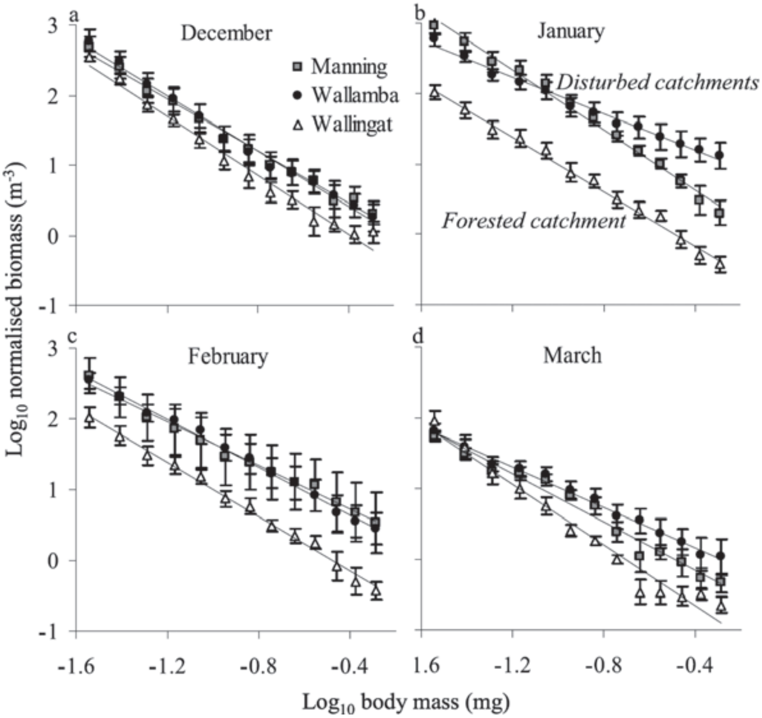


Figure 3.4 The average Normalised Biomass Size Spectrum (NBSS) for zooplankton caught in a 100 µm mesh net in three temperate estuaries, during four summer months (after Moore and Suthers 2006).

estuaries with rural catchments and nutrient enrichment, versus the small estuary with a forested catchment (Figure 3.4). The more pristine estuary often had a steeper slope and lower overall biomass, which we attribute to the greater water clarity allowing visual-feeders such as fish to predate the larger zooplankton and thus steepen the slope (Figure 3.3).

The role that zooplankton play in assimilating algal biomass was shown clearly in work conducted in Dee Why lagoon – a small coastal lake in the northern beaches area of Sydney. The lake is closed off from the ocean for long periods of time, which removes the influence of tidal flushing and enables biological responses to rainfall to be examined. We sampled nutrients, phytoplankton and zooplankton at regular intervals before and after a large rainfall event, after a prolonged summer dry period. Nutrients (ammonia and oxidised nitrogen) significantly increased the day after initial rainfall, before returning to pre-rainfall conditions within 5 days. In response, phytoplankton

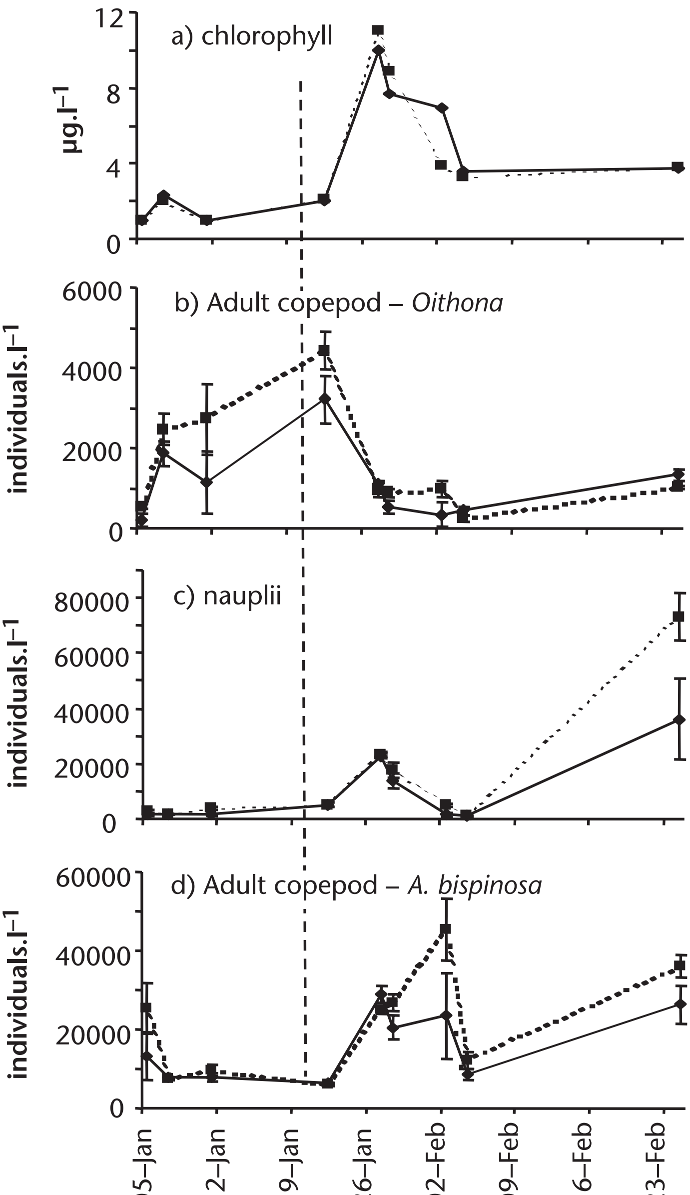


Figure 3.5 Changes to average plankton at two sites within Dee Why lagoon over the study period. Vertical dashed lines indicate the main, initial rain event. a) Phytoplankton biomass (μg chl-*a*.L^{-1}). b) *Oithona*, an adult copepod, which doubled in abundance within 48 hours. c) Copepod nauplii. d) Adult *Acartia bispinosa*.

biomass grew 10-fold within a week after the initial rainfall and declined to near original levels 2 weeks later (Figure 3.5a). Blooms of diatoms followed the rainfall within a week, which returned to pre-rainfall levels within 2 weeks. It was clear that zooplankton, which increased in response to the higher phytoplankton concentrations, were responsible for the rapid decline in phytoplankton. However, some zooplankton responded within a day with two fold increase in the adult stages of the calanoid copepod *Oithona* sp. (Figure 3.5b), followed a week later by nauplii (Figure 3.5c) and adult *Acartia bispinosa* (Figure 3.5d). The influx of adult zooplankton into the water column was presumably from resting populations that were previously under sampled by our plankton net. The zooplankton community returned to the initial state by 2 weeks and then matured to a centric diatom-*Acartia* dominated population after 5 weeks.

3.8 IMPACT OF REDUCED FRESHWATER INFLOW ON THE PLANKTON OF SOUTHERN AFRICAN ESTUARIES

Increased freshwater removal due to population growth and industrialisation has resulted in a decrease in the amount of freshwater inflow into southern African estuaries. From a biological perspective, the reduction in freshwater inflow into estuaries has led to a decrease in the total phytoplankton primary production, because freshwater inflow provides new nutrients for the growth of phytoplankton. The decline in riverine inflow into estuaries has also been associated with changes in the species composition and distribution of both invertebrates and fish (Froneman 2002a, b; Mallin and Pearl 1994). The impact of reduced freshwater inflow on the food web dynamics of estuaries is poorly understood, despite the implementation of environmental flow regulations in many cases.

The Kasouga estuary is a medium-sized temporarily open/closed estuary located within the warm-temperate region along the southern African coastline. During the dry season, the estuary is separated from the sea by the presence of a sandbar at the mouth. Following periods of high rainfall and freshwater run-off, the volume of the estuary rises until it exceeds the height of the sandbar. Breaching then occurs, which culminates in riverine conditions predominating throughout the system. The development of a sandbar within weeks of the breaching event due to long-shore drift results in the estuary rapidly being closed off from the sea. During the subsequent closed period, seawater inflow is provided by wash-over during spring high tides or during severe storms.

The Kasouga estuary has a surface area of 28 hectares and the catchment area is estimated at 39 km^2. The estuary is approximately 2.5 km

in length and is generally shallow (with a depth less than 2 m). Previous investigations have shown that the nutrient status of the estuary ranges from an oligotrophic (Redfield ratio of N:P approximately 7:1, Section 2.2) to eutrophic system (Redfield ratio approximately 14:1). A shift in the nutrient status of the estuary is determined by freshwater inflow into the system. The increase in macronutrient availability following freshwater inflow into the estuary coincides with dramatic increases in the phytoplankton primary productivity and zooplankton biomass (Froneman 2002b). The Kasouga estuary therefore, represents an ideal system to assess the impact of reduced freshwater inflow on the estuarine food web dynamics.

This study was designed to investigate the influence of changing freshwater inflow on the food web dynamics of southern African estuaries. Chlorophyll-*a*, primary production and zooplankton (larger than 200 μm) grazing studies were conducted monthly for a year in the Kasouga estuary, in the upper, middle and lower reaches of the estuary (Box 3.7).

Four separate periods of rain, and resultant inflow to the estuary, coincided with an increase in total phytoplankton biomass and productivity (Figure 3.6a, b). There were no significant spatial differences in plankton between the various regions of the estuary and therefore results have been pooled. The mean total phytoplankton biomass and daily phytoplankton production during study ranged between 0.9 and 6.3 mg chl-*a*.m^{-3} and

BOX 3.7 HOW SAMPLING WAS CONDUCTED IN THE KASOUGA ESTUARY

Chlorophyll-*a* biomass was determined by filtering a precise volume of water through a filter and extracting the chlorophyll into acetone, which is then analysed by fluorescence. Phytoplankton production ('primary' production) was determined by incubating a water sample with carbonate labelled with the radio-isotope C14, to determine how much is converted into phytoplankton. To determine zooplankton biomass at each station, net tows were made at night using a WP-2 net with a mesh size of 100 μm. The net was fitted with a flow meter to determine the amount of water filtered during each tow. Zooplankton biomass, expressed as mg dry weight per unit volume (mg dwt.m^{-3}) was converted to carbon equivalents assuming a carbon content of 40% dry weight (Froneman 2002b). Zooplankton grazing impact was determined employing a radio-isotope label (Mallin and Pearl 1994). Mass specific ingestion rates of the zooplankton were calculated by dividing the zooplankton biomass (in terms of carbon equivalents) by the zooplankton ingestion rate. A chl-*a*: carbon ratio of 50 was assumed.

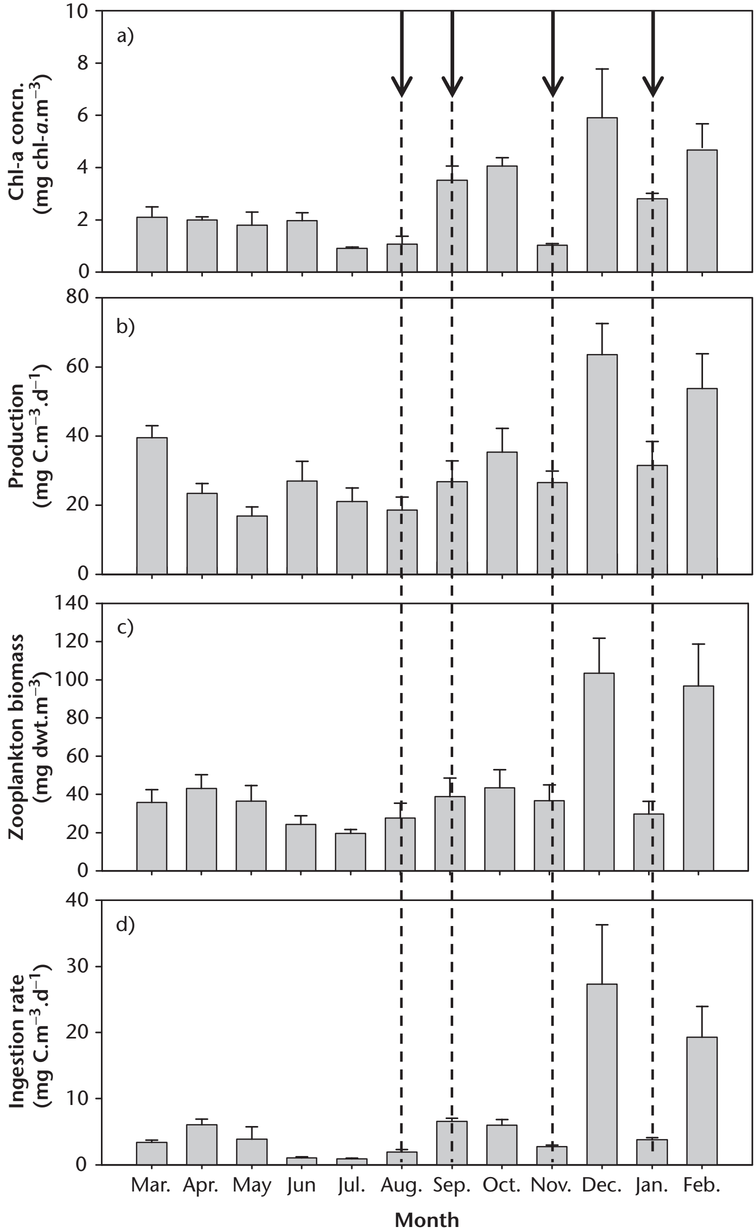


Figure 3.6 Effects of rainfall on an estuary's plankton, in the temporarily open/closed Kasouga estuary situated along the south-eastern coast of southern Africa. Arrows indicate periods of rainfall; a) total phytoplankton biomass, b) productivity, c) zooplankton biomass, and d) zooplankton community ingestion rates. Error bars are standard deviation.

between 16.9 and 63.5 mg C $m^{-3}.d^{-1}$, respectively (Figure 3.6a). A distinct temporal pattern was evident with the highest biomass and production values (generally greater than 3 mg chl-*a*.m^{-3} and greater than 40 mg C $m^{-3}.d^{-1}$) recorded following freshwater inflow into the estuary (Figure 3.6a, b). In the absence of freshwater inflow, total phytoplankton biomass was always less than 1.5 mg chl-*a*.m^{-3} and daily phytoplankton production always greater than 25 mg C $m^{-3}.d^{-1}$.

The inflow and increased nutrients permit large phytoplankton cells (greater than 5 μm) to dominate total phytoplankton production (Froneman 2002a). In contrast, under conditions of reduced/no freshwater inflow, nutrients are limiting and total production is dominated by picophytoplankton (less than 2 μm) which have a better surface area:volume ratio to facilitate nutrient uptake (Froneman 2002b). These tiny phytoplankton are too small to be eaten by many of the estuarine copepods (grazers). Generally zooplankton are unable to feed efficiently on phytoplankton cells less than 5 μm. Total zooplankton biomass in the Kasouga estuary demonstrated a strong temporal pattern with the highest values (greater than 45 mg dwt.m^{-3}) recorded following periods of freshwater inflow into the estuary. Total zooplankton biomass in the absence of riverine inflow into the estuary ranged between 19.5 and 43.5 mg dwt.m^{-3} (Figure 3.6c). Zooplankton community ingestion rates during the study ranged between 0.8 and 27.3 mg C.d^{-1}. The highest ingestion rates were recorded following freshwater inflow into the estuary (Figure 3.6d). This increase in the zooplankton biomass and grazing activity of the zooplankton following periods of freshwater inflow into the estuary can be related to increased food availability (chl-*a*) and the availability of their preferred food particle size (greater than 5 μm).

The shift in the size composition from a community dominated by large phytoplankton cells during run-off, to one dominated by small cells during dry spells, has important implications for the feeding ecology of the zooplankton in the estuary. Copepods require 30% body carbon per day to meet their basic metabolic requirements. Results of the grazing studies indicated that the mass-specific ingestion rates of the zooplankton under conditions of reduced freshwater inflow was generally equivalent to less than 30% body carbon per day. These data suggest that carbon derived from the consumption of phytoplankton was insufficient to meet the basic metabolic requirements of the zooplankton and that alternative carbon sources are used, including detritus and/or carbon derived from the microbial loop (see Chapter 2, Figure 2.3). In contrast, when freshwater inflow into the estuary occurs, phytoplankton-derived carbon is sufficient to meet all the carbon requirements of the zooplankton.

Therefore, based on the Kasouga estuary study, it is likely that reduction in the amount of freshwater inflow into estuaries is likely to result in a decrease in the size structure and productivity of both phytoplankton and zooplankton communities. Extraction of freshwater will exacerbate this effect, with a shift to clear coastal water and less-productive planktonic food web. In the absence of freshwater inflow into estuaries, much of the phytoplankton production appears to be unavailable to the zooplankton due to feeding constraints. The unfavourable size structure of the phytoplankton community within freshwater-deprived estuaries is likely to decrease the trophic efficiency within these systems.

Clearly, plankton grazers are very discerning in what and when they can eat. Managers of environmental flow regulations should therefore use plankton communities as sentinels of the necessary flow and production for normal estuarine production. Too much nutrient or anthropogenic nutrients (dominated by N and P, with low Si) would lead to eutrophication and blooms of less-palatable or less-productive phytoplankton, with socio-economic problems.

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Chapter 4

Sampling methods for plankton

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4.1 INTRODUCTION TO SAMPLING METHODS

When preparing for sampling, time invested in formulating unambiguous questions, and appropriate methods and analyses, is time well spent. A pilot study – even an afternoon of sampling – will hone your proposal. You must also decide to what degree are the samples to be analysed (for example, just biomass, or by size, or to phylum level, or right down to species?). Many issues can be addressed by using biomass, size or classifying plankton into broad taxonomic groups. Try to imagine the data and even the graph that you seek (that is, your goal), and then plan a program that will put data onto that graph.

There is no single, generic sampling method – the method chosen must suit the question. Plankton is not distributed uniformly throughout the water, but has a patchy distribution in both space (vertical and horizontal) and time (between day and night, winter and summer). This means, for example, that sampling with a particular size of mesh, or during the night, or during the ebb tide will influence the results and the interpretation. Details and examples are provided in this chapter on:

- determining a robust sampling design in relation to the objectives
- the observation, preparation and quantification of plankton samples
- the fixation and preservation of plankton samples.

Defining your question is perhaps the most important, and most difficult, issue of plankton studies because it requires you to consider exactly what information your organisation requires in the short and long term. Once your question has been defined, the proposed statistical analysis that answers your question must be considered before data collection even begins. Your question, and the proposed statistics, should be compared – which should provide a logistically feasible sampling design. If in doubt then get advice, because much sampling effort has been wasted in the past by not considering the final analysis. Conflicting advice on statistics is typical, and it is up to you to rationalise differing views. Once your question has been targeted, re-phrase it as a testable hypothesis (see Box 4.1), and then discuss it with your colleagues, to determine if it is achievable. In the past, poor sampling regimes, such as blindly collecting water samples on the first Monday of every month without reference to rainfall pattern or tide or using control sites, have led to results being almost useless.

BOX 4.1 THE SCIENTIFIC METHOD

We generally work by the scientific method, where an observation or a contention is expressed as a model. This model is formally expressed as a hypothesis, but is tested in the form of a negative or null hypothesis (because we can never completely prove a model, but it only takes one test to disprove it). We then test if this null hypothesis is true or not, and refine our model accordingly. We determine if the significant difference among our samples is so great that there is only a 5% probability (i.e. $p = 0.05$) that such a difference could have occurred by chance.

While this approach is useful in some aspects of ecology, most other sciences accept that a null hypothesis is often illogical and that a realistic alternative or null model is philosophically more sound. Perhaps you may have no particular 'scientific' goal, other than to commence monitoring. A better null hypothesis (than saying there is no significant difference) would be that 10% more nutrients may increase algal productivity by 10%, rather than zero effect. It is our responsibility to ensure that the null model is a sensible alternative. Our arbitrary use of the 5% probability criterion ($p = 0.05$) is also fairly extreme, when 10% or even 25% may be more conservative (depending on the variability of your data, or statistical power). By rejecting the null model with only strong evidence, there is a risk of retaining the null model when it is incorrect (termed a 'type II error'). The long-term implications of wrongly concluding 'no significant impact' are more severe (such as a loss of species) than wrongly concluding 'a significant impact' (which is a nuisance).

There is great value in attempting to assimilate old data or data collected using less-than-perfect sampling methods. This is because there is a gradual, declining standard of our environment, which is not easily noticed, but a comparison of water quality over decades would sometimes be shocking. There is great value in incorporating a flawed study into a good sampling design, to assess the earlier flaws within a context. It is important that, if doing so, the uncertainty or relevance of the data is understood and discussed. Some data should not be used, including some nutrient data where methods are not documented or have changed considerably.

Salinity and temperature are key environmental traits that place the plankton into a context. The physical structure of estuaries must be measured at every station, as the water mass or vertical stratification can influence plankton communities (see Chapter 2, Figure 2.9). For example, a vertical profile of salinity and temperature at a number of sites can enable you to assess whether the waterway is stratified, horizontally or vertically (see Chapter 2, Figures 2.9, 2.10). Physical and chemical water properties vary daily, seasonally and yearly because of natural seasonal cycles, daily fluctuations in the physical environment (such as tides) which determine the plankton community. Estuarine plankton communities vary according to the salinity of the waterway. At the most upstream reaches with salinities between 0 and 3, the community consists mainly of freshwater taxa. At salinities between 3 and 20, the communities are a mixture of freshwater taxa and marine taxa, with an increasing dominance of marine taxa as the salinity increases.

4.2 DEALING WITH ENVIRONMENTAL VARIABILITY

4.2.1 Independent samples

Good sampling design requires that each level of your design, and each replicate sample, is independent of each other. Dividing a plankton sample in half is not a replicate. For example, samples should not be collected simultaneously – and replicates sampled immediately downstream of another sample are not independent. The paired nets of a bongo net (see Section 4.7) are not independent replicates. Moving the vessel away from the initial sample does satisfy the requirement of independent sampling.

To assess water quality you may need an additional level of analysis – that of an independent estuary or lake. This is because the water body of interest may be changing throughout all bays and coves due to changes in its catchment (of major concern to a manager), or due to global or regional

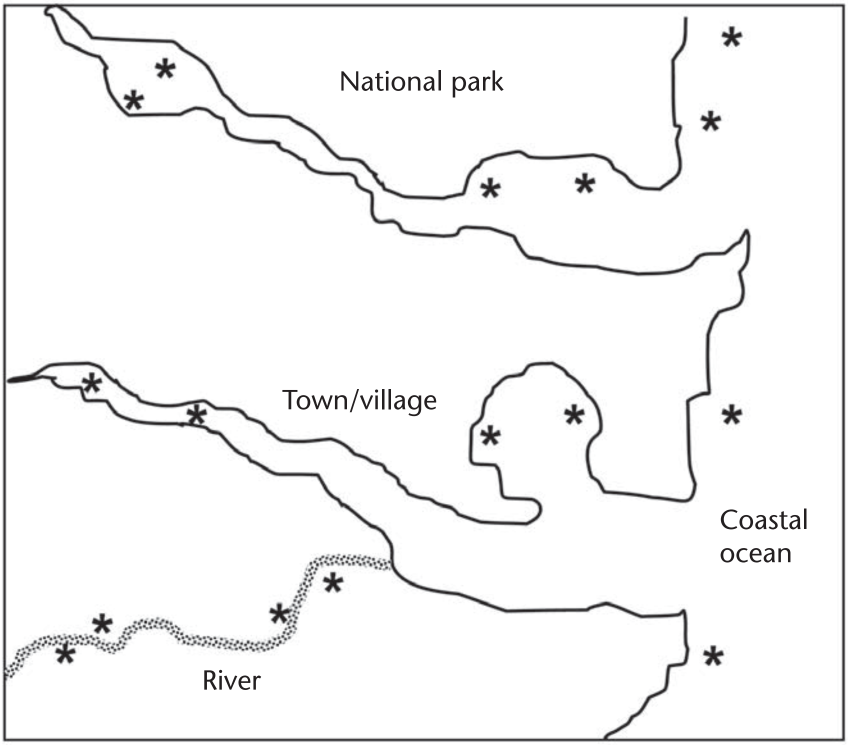


Figure 4.1 Detection of an effect at independent reference sites. A possible monitoring design of an estuary or river, illustrating the importance of replicating sites in each region (with a replicated sample from each), and possibly an external reference estuary. The external site is to ensure that changes in your estuary are not due to some global or climate phenomenon. The external data could be another municipality's monitoring program – it does not have to be pristine. Perhaps the town or village was wishing to install an artificial wetland or perhaps fencing along one of the rivers. This design could assess the environmental cost–benefit. Your sampling needs to have replicate sites to ensure that the changes you are observing are not just peculiar to one site and unrelated to the wetland or fence.

changes (also of concern, but not within a manager's mandate). Consequently a parallel sampling program should be conducted in two related or similar water bodies (Figure 4.1). It is difficult to convince managers to invest funds outside their constituency, despite the need to benchmark your own investigation. One approach is to do the regional comparison during a particular summer month only – in a separate analysis – or by collaborating with other groups.

4.2.2 Spatial and temporal scale

Many ecological processes maybe relevant at the small scale (minutes or metres) or at the large scale (years or tens of kilometres). Water-quality managers generally operate within a 1- to 20-year timeframe and a 1- to 20-kilometre spatial scale. Consequently, you will find in this section reference to a sampling hierarchy: from the level of sub-sample to site, to embayment, to water body; and from the level of day, month and perhaps year. This sampling strategy is particularly appropriate in marine ecology for the analysis of variance (ANOVA), which partitions the variability among the factors and their levels (see Box 4.2). Despite some constraints, this approach explicitly lays out your sampling proposal, defining a hierarchical sampling design (for example, estuaries, months, days, sites and replicate tows),

BOX 4.2 VARIANCE, PATCHINESS AND STATISTICAL POWER

The sum of the squared differences between each observation and the overall mean value ($\bar{x}$) is known as the variance (s^2), and the square root of the variance is termed the standard deviation (s). The standard deviation may be compared between ponds or days or species by a coefficient of variation (CV), expressed as ($100 \times s/\bar{x}$). It is the variance that determines confidence limits and significant differences. An estimate of the number of replicates (n) needed to be within 5% of the average value may be calculated from a pilot study by $n = (s/(0.05 \times \bar{x})^2$ (Kingsford and Battershill 1998, p. 53).

Biological and physical processes can promote patchiness or clumping, such as cell division, or cell buoyancy. A random distribution is a mixture of a completely uniform distribution of animals (with low or zero variance), with a completely clumped distribution (with very high variance). The degree of clumped distribution is described as patchiness, which can vary among species or in space (such as at the centimetre, metre and kilometre scales, such that there are patches of patches, rather like suburbs, towns and cities).

The concept of statistical power pervades any environmental impact assessment. The cheapest approach to an environmental impact is to take just two replicates in an impacted and non-impacted area. Inevitably, the natural variability will swamp any difference between the two areas, and you would wrongly conclude no significant impact (a type II error). Such a flawed comparison would be an example of low statistical power, because of low replication or high variability. Managers need to be wary of quick and cheap assessments – and also be aware of any attempts to avoid environmental responsibility for an impact, under the guise of natural variability.

even if you choose not to use this particular analysis (see Kingsford and Battershill 1998). Regression and correlation are useful methods to further test your findings.

4.2.3 Variance, sample size and replication

Natural variability underscores all biological sciences, from evolution to ecology. Variability is what determines the importance of an average value, and of the changes that you may discover. For example, finding that the average summer chlorophyll values in your pond increase over 10 years from 0.5 to 2.0 μg per litre may not be as important if the range during each summer was 0.5 to 10 μg per litre. For this reason ecologists are often concerned with the degree of variability as well as the average value (see Box 4.2).

Variation associated with the natural patchiness of plants and animals can be 10- or 100-fold greater than the variation in physical characteristics, such as sediment type or water temperature. The degree of variation is often in proportion to the average value (that is, a large value has a greater capacity for variation than a small value) and, in part, to the spatial and temporal range (samples taken at metres or seconds apart vary less than those taken at kilometres or months apart). Variability may occur at temporal scales of less than a few hours and at spatial scales less than hundreds of metres and most questions for water-quality management occur at scales greater than this.

As a first step, we often sample large volumes of water with a plankton net or pole sampler that integrates, or mixes, this small-scale variability. Plankton net tows are different to many other kinds of ecological sampling such as benthic cores, quadrats, fish counts or bottle samples of water, because a tow over 5–10 minutes integrates many fine-scale patches. Therefore the variance among replicate tows is often small (Box 4.3) and while we may generally collect three or four replicate tows, you may need to only sort and identify two. Nevertheless, we do need to know the degree of variability at the scale of our sampling device, and at each and every level above. To further pool all the samples of a particular region would become pointless – because the value and statistical power of just two replicates exceeds one pooled sample.

Your pilot study may indicate the need to consider additional factors, such as replicate days or months, to partition the variability. These could otherwise overwhelm your variable of interest (such as an estuary with, or without, sewage treatment). Normally every factor of your analysis should be replicated (that is, 2 or 3 replicate days). By inserting additional factors, samples numbers and costs can quickly escalate. However, without partitioning the variability, you would have to take many more replicates at the level of your sampling unit, and the statistical power would be lower.

BOX 4.3 WHERE PLANKTON VARIANCE MAY BE EXPECTED

Relative coefficient of variation (CV) of plankton within an estuary (if all other factors are constant). The table is based on our experience with towed nets (100–500 μm mesh) of at least 3 minutes duration (that is, integrating many fine-scale patches) and should be used as a guide only. The number of stars represents the approximate variability in plankton that could be expected by sampling, for example, at one site before and after rain. Patchiness (variability) in time is generally greater than spatial patchiness, but sampling over time takes more organisation and effort.

	Factor	Relative CV
Temporal:	Before/after rainfall	*****
	Day/night	****
	Morning/afternoon	*
	Between flood/ebb tides	****
	Among days	*
	Among weeks	**
	Among seasons	*****
	Between two years	*
Spatial:	Among estuaries	***
	Among habitats within an estuary	**
	Among sites within a habitat	*
	Within a site (i.e. among replicates)	*
	Among sub-samples	*
	Surface/depth (between 0 and 5 m)	**

We may also need to quantify variability at the level of our sampling device by taking replicate samples. The number of replicates needed is in proportion to the variability, which is frequently determined by a pilot study. Instead many scientists guess by 'taking two, three or four samples', which, for many plankton studies, may actually be appropriate, providing there is a suitable hierarchy of sampling levels. In summary, your final design will depend on whether you are planning a baseline study, an impact study, a monitoring study or to determine patterns and processes (Kingsford and Battershill 1998).

Variability in plankton samples can be dealt with by three methods:

- integrate (taking larger samples)
- stratify (recognise regions or days to block your data)
- replicate (increase your base sample size in proportion to the variability).

You may consider a cost–benefit analysis, whereby you balance the competing needs of a limited budget, increased replication and/or inserting

levels into your sampling design, or integrating variability with larger samples. There are formal ways of balancing these competing needs explicitly in terms of dollars to variance (see Kingsford and Battershill 1998). For most plankton studies needing identification, the major costs are the sorting and analysis, rather than the collection costs.

4.3 TYPICAL SAMPLING DESIGNS: WHERE AND WHEN TO SAMPLE

An established monitoring program of water quality should have the capacity to be incorporated into an unexpected impact assessment. A robust monitoring program can account for the intrinsic, natural variation, and statistical or graphical methods can partition natural and artificial variability among your sampling sites. Data collected over long periods of time can be used to explore the response by plankton in time and space and to infer a process. It is also possible to manipulate the environment in an experiment in a way that specifically adds or subtracts a component that you believe to be important in influencing water quality.

Temporal variation must be accounted for – despite the factors of interest often being spatial (impacted versus control sites). Day/night effects incorporate a large proportion of zooplankton variability, due to diel vertical migration, emergence into the plankton of epibenthic groups, selective tidal stream transport and net avoidance. Significantly more and larger zooplankton is caught at night, but this community may have a significant epibenthic component, which may not be useful to your question. Whether you sample during the day or at night is not crucial, so long as you are consistent and avoid the effects of dawn and dusk. Similarly, you should consistently sample the ebb or flood tide, depending on your question. If you sample only on the ebb tide, you will be sampling water that has spent at least the past 6 hours in the estuary, and thus reflects estuarine conditions. Because plankton can rapidly increase over days and weeks, a robust plankton sampling design should include daily to weekly variation. If the seasonal component is not important to you, then you could just sample the midsummer months, on an annual basis.

Choose sites on the basis of logistics and safety, and avoid areas with conspicuous fronts and foam lines. Pay careful attention to tidal characteristics, estuarine flow, wind strength and direction, which can influence plankton abundance. Some sites characteristically support a bloom (such as Berowra Waters; see Section 3.2).

The bathymetry where sampling occurs may have a large effect on plankton composition in lakes and estuaries. Such areas are often well mixed

from top to bottom and an oblique – or near-surface tow – is adequate. Vertical phytoplankton hauls or pole samplers will also mix or ignore any vertical structure. In general the effect of depth is ignored in sampling areas <5 m depth, provided the sampling protocol is consistent. Ensure that you record at least the temperature and salinity at every station.

You may sample plankton at point stations, or along transects, or at a grid of stations. The survey method used will depend primarily on tidal currents of the inshore sub-tidal habitat, and on study objectives. A transect of stations is appropriate if an alongshore or across-shore gradient in phytoplankton is suspected. A grid of stations should be used if there is large spatial homogeneity in habitat unit.

How often should you sample? If plankton monitoring is your goal, then sampling every 2 to 3 days (during a similar phase of tide), on each of two to three midsummer months is a good start. Representative regions should be sampled with at least two stations in each, with two to four replicate, depth-integrated samples at each station. To monitor the effect of rainfall and run-off does require a degree of opportunistic sampling (Moore *et al.* 2006).

Alternatively, if plankton impact assessment is your goal, then a particularly powerful sampling design is the 'beyond BACI' – a before, after, control, impact assessment – at multiple control locations and at multiple times (Kingsford and Battershill 1998). This is an ANOVA-based sampling design for when a development is anticipated and sampling can be conducted before and after the impact, along with multiple control locations (Figure 4.1). Without any pre-impact data, the impacted site can only be compared with control sites, which themselves will be naturally variable – reducing the chance of detecting an effect. The impact of rainfall events is particularly relevant in estuaries, where the effects of urban run-off continue long after the initial flood. The analysis is sophisticated and requires statistical advice or at least guidance – but an impact would be assessed by adapting a survey design.

4.4 MEASUREMENT OF WATER QUALITY

Estuarine water quality is dependent on a number of factors, such as loads of nutrients and sediments to the system, recycling of nutrients within the system, reworking of sediment and other integrating factors within the system (such as assimilation, flushing and light penetration). Water-quality parameters can be separated into those that are toxic to organisms at certain levels and those that have indirect effects on organisms by changing the nature of the system, such as nutrient overloading. Water quality can be

determined using a variety of means, including direct measurement of specific variables, such as nutrients, or by measuring other variables, such as phytoplankton biomass or biodiversity. Phytoplankton biomass is a useful indicator because phytoplankton integrate many water-quality attributes over a variety of time scales and, although temporally and spatially variable, are less so than factors such as nutrients.

Water temperature (T), along with salinity (S), characterises the 'T–S signature' of water habitats (Box 4.4). The actual differences in T and S may be physiologically trivial, yet minute changes of just 0.1°C in temperature or 0.01 in salinity can be the planktonic equivalent of moving from a desert to a rainforest (see Figures 2.9, 2.10).

BOX 4.4 ELECTRONIC DETERMINATION OF SALINITY

Salinity used to be determined chemically, such as from the concentration of chlorine ions – which uniformly account for 55.0% of total ions. A kilogram, or nearly a litre, of seawater typically contains about 35 g of salts (or 3.5% weight for volume), and therefore has been expressed as 35 ppt. Today, one of the most common methods of estimating salinity is by its electrical conductivity. This modern method of salinity is a ratio of two electronic signals, so today there are no units for salinity ('the salinity was 35 last week'). For a given temperature, conductivity of water varies linearly with ion concentration – making measurement of electrical current between two submerged electrodes a convenient measurement (Figure 4.2a, b). Alternatively, salinity can be measured by inducing an electric field around the sensor, which is linearly proportional to the concentration of ions. Particular attention should be paid to this type of sensor as spuriously low readings will be recorded if it touches the side of the bucket, or even seagrass.

A simple, but coarser, measurement of salinity is the refractive index of water, which is measured with a portable refractometer using just a few drops (Figure 4.2c, d). The refractometer is calibrated for a direct read-out of S at 20°C. Salinity may be expressed in parts per thousand (ppt), or practical salinity units (psu), or usually without units (as the electrical method is actually a ratio). Unlike temperature, salinity is ecologically conservative parameter, and so it is an excellent indicator of circulation in an estuary. Together with water pressure, temperature and salinity determine the density of sea water. The density of pure water at 15°C is 1000 kg per m^3 (that is 1 kg per litre), while warm sea water at 25°C and a typical oceanic salinity of 35 is about 1023.3 $kg.m^{-3}$ (that is, 1.023 $kg.L^{-1}$). The density is therefore expressed as rho ($\rho = 1.0233$). Oceanographers abbreviate this to sigma (in this case, $\sigma = 23.3$; same units by convention).

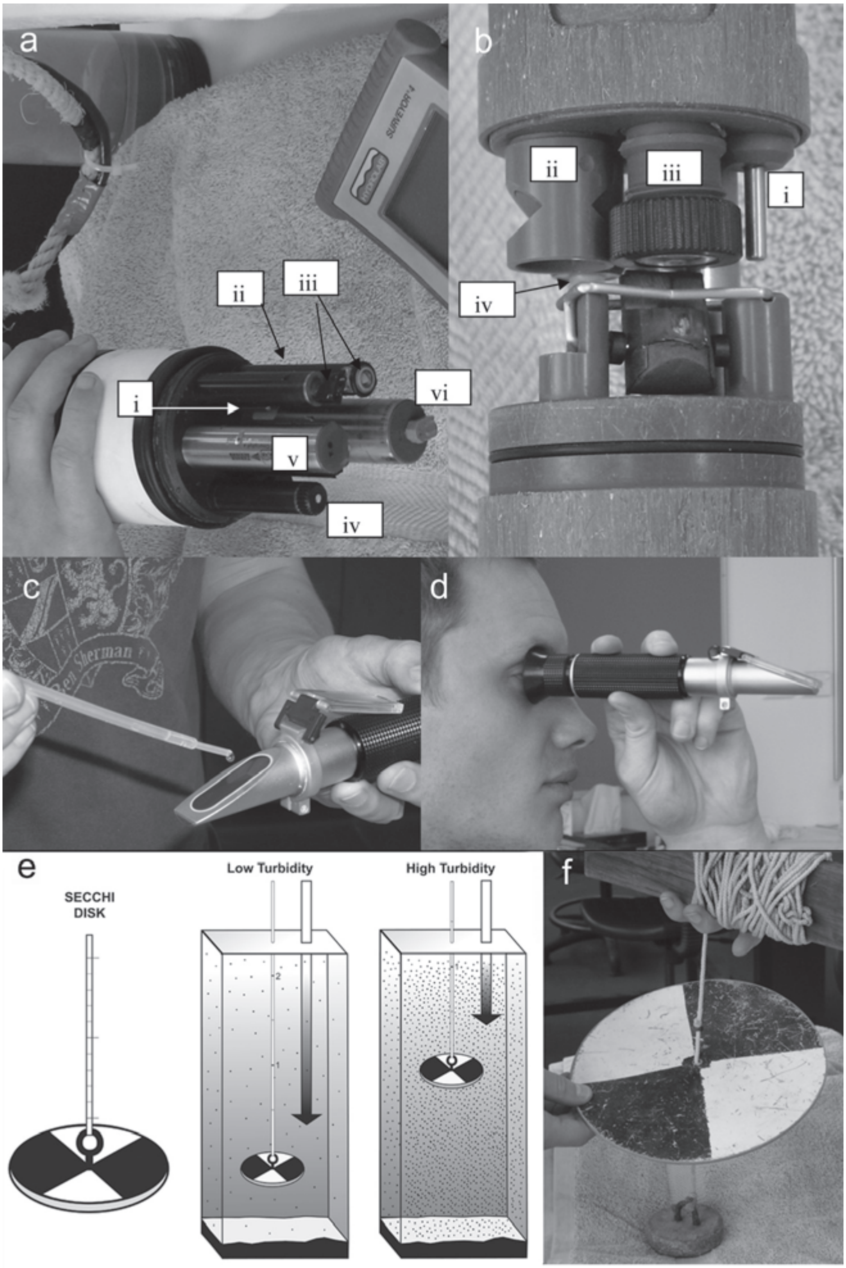


Figure 4.2 a), b) Typical commercial CTD probes – (i) temperature, (ii) conductivity, (iii) dissolved oxygen and associated stirrer, (iv) pH and reference electrode (partially hidden), (v) turbidity, (vi) chlorophyll; c), d) using a refractometer; e), f) a Secchi disc and its deployment for measuring turbidity.

Modern probes may have a chlorophyll fluorescence sensor (Figure 4.2a). This instrument shines a blue light into the water, which, in turn, causes the chlorophyll to fluoresce (that is, the chlorophyll molecule emits a photon). Once calibrated with actual extractions, as outlined above, the fluorescence is roughly proportional to the actual biomass of chlorophyll. The advantage of fluorescence over absorbance is that it only needs in situ concentrations – no extraction into solvents is necessary. The disadvantage is that many factors influence fluorescence, and the signal is at best ±50% precise. Other commercial fluorescence sensors make in-situ measurements of other pigments contained within phytoplankton cells. Examples include sensors that measure phycocyanin presence (that indicate the amount of cyanobacteria (blue-green algae) present in freshwater environments) or phycoerythrin (to determine cyanobacterial and cryptophyte presence in marine waters).

Turbidity refers to the interference of light by suspended matter, soluble coloured organic compounds or plankton in the water. The measurement of turbidity is used as an indirect indicator of the concentration of suspended matter, and also is important for evaluating the available light for photosynthetic use by aquatic plants and algae. One method of measuring turbidity is with an electronic transmissometer, which measures light attenuation in water optically, yielding a percentage transmittance. A much simpler, traditional method is to use a Secchi disc (Figure 4.2e, f). A Secchi disc is a black and white disc that is lowered in water to the point where it is just barely visible in order to measure the depth of light penetration (if you can see the bottom of the water body then it is not possible to measure a Secchi depth). Light penetration is progressively reduced by absorption with increasing water depth. Primary production is generally considered to take place to depths at which more than 1% of surface light is available.

Total suspended solids (TSS) refer to the concentration of suspended solid matter in water. TSS is measured by weighing the undissolved material trapped on a 0.45 μm filter after filtration. The constituents that pass through the filter are designated total dissolved solids (TDS) and are comprised mainly of ions such as iron, chloride, sodium and sulfate. It should be noted that there is a direct proportional relationship between suspended solids and turbidity. The solids in suspension may include sediment or detrital particles and plankton.

Dissolved oxygen (DO) is the traditional and ubiquitous indicator of aquatic health. It determines the ability of aerobic organisms to survive and, in most cases, higher dissolved oxygen is better. The concentration of dissolved oxygen depends upon temperature (an inverse relationship), salinity, wind and water turbulence, atmospheric pressure, the presence of oxygen-demanding compounds and organisms, and photosynthesis. Of these, DO is

introduced into the water column principally through re-aeration (simple mechanical agitation by wind) and through photosynthesis. DO is typically around 4 to 8 $mg.L^{-1}$, or reported as percentage saturation, when 100% is in equilibrium with the air. Therefore high percentage saturation occurs during the day due to algal photosynthesis, and low (hypoxic, less than 1.5 $mg.L^{-1}$ DO) or anoxic water (around 0 $mg.L^{-1}$) occurs late at night due to respiration and decomposition. Even at 100% saturation, warm salty water holds less DO than cool fresh water. Dissolved oxygen deficit is the difference between the capacity of the water to hold oxygen and the actual amount of DO in the water (the converse of percentage saturation). A large deficit is an indicator of some oxygen demanding stress on natural waters, while a low deficit is an indicator of generally unstressed conditions (DO gives no indication of possible toxic contamination).

pH is a measure of acidity or alkalinity of the water. High pH indicates that the water is alkaline and low pH indicates that the water is acidic. Generally, pH exhibits low variability in coastal situations due to the high buffering capacity of seawater. Departures from the normal range of 7–9 are therefore especially significant (the pH scale is logarithmic). Low pH occurs following rainfall events on areas with exposed acid sulfate soils. The sulfuric acid run-off from these exposed soils can cause direct mortality of biota, as well as a variety of sub-lethal effects. Acid run-off also influences the chemistry of estuaries and can also damage infrastructure.

Biochemical oxygen demand (BOD) is an indirect measure of biodegradable organic compounds in water, and is determined by measuring the dissolved oxygen decrease in a controlled water sample over a 5-day period. During this 5-day period, aerobic (oxygen-consuming) bacteria decompose organic matter in the sample and consume dissolved oxygen in proportion to the amount of organic material that is present. In general, a high BOD reflects high concentrations of substances that can be biologically degraded, thereby consuming oxygen and potentially resulting in low dissolved oxygen in the receiving water. The BOD test was developed for samples dominated by oxygen-demanding pollutants such as sewage. While its merit as a pollution parameter continues to be debated, BOD has the advantage of being used over a long period.

4.5 SAMPLING METHODS FOR PHYTOPLANKTON

You should choose a method based on your question, the precision required and your budget. If your purpose was to collect a sample to determine what species were present in an algal bloom, and not for any comparative

purposes, it is possible to collect three samples, mix them together in a bucket and then take a sub-sample for counting. This sub-sample will provide an indication of the average counts, but will give no indication of the variation between the samples.

Visual assessment is the least expensive way to monitor phytoplankton – by estimating phytoplankton abundance based on water colour, Secchi depth, area of bloom or from a satellite image. You can also make net collections (20 µm mesh; see Figure 4.4, Section 4.6), to concentrate rare species. Net collections of phytoplankton are suitable for larger cells, such as some diatoms, but the bulk of phytoplankton in the sea and in rivers is in the less than 20 µm fraction and even in the less than 2 µm fraction. Consequently, a plankton tow is regarded as a qualitative measure due to avoidance and particularly extrusion of particles through the mesh.

Quantitative samplers include surface water samples, which are collected by dipping a well-rinsed bucket over the side of the boat. A sample may be collected from the shore or bank with an empty sample jar on a pole. Integrated samples are usually taken from the surface to 3 m depth or more. These samplers can be made from a 2–5 cm diameter PVC or hosepipe (Figure 4.3a). In rivers with extensive rushes and mud banks, a Taylor sphere sampler (TASS) is a simple and ingenious device (Figure 4.3b, Hötzel and Croome 1999). Both samplers are operated in different ways but work on the principle that an integrated sample is taken through the photic zone of the water column. The entire sample is then released into a clean bucket – repeated up to three times – and a 100 mL sub-sample is then removed from the bucket and preserved for phytoplankton identification.

Water samples from specific depths can be collected using diaphragm pumps or water bottles, such as 1.7 L Niskin bottles. Water bottle casts (‘hydro-cast’) can be conducted using a rope over the side of a boat, and a heavy metal ‘messenger’ then slides down the rope to close the bottle.

At least two replicate water samples should be collected at each station or depth, and their unique numbers recorded on the field data sheet. An extra water sample from each hydro-cast should be retained in case of laboratory mistakes. Label each bottle with a unique identifying number (inside and outside) for the laboratory. A pad of self adhesive labels is useful, such that the same number can be used on the various samples for nutrients, chlorophyll, phytoplankton and zooplankton and the data sheet.

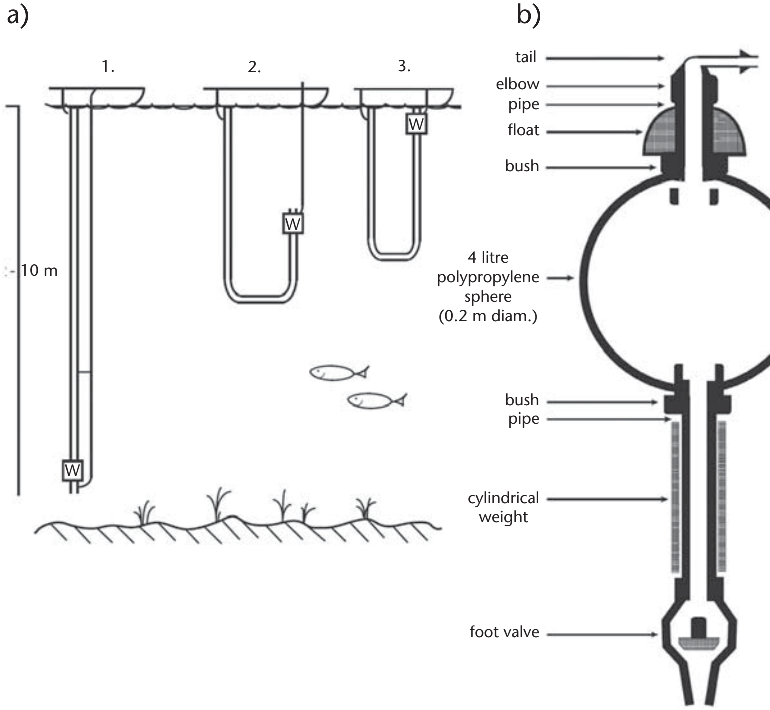


Figure 4.3 Depth integrated samples of the water. a) Hosepipe sampler. b) Taylor sphere sampler (TASS).

4.6 ANALYSIS OF PHYTOPLANKTON SAMPLES

Phytoplankton samples collected using appropriate quantitative sampling methods can be analysed in the laboratory by various counting methods or by the measurement of chlorophyll-*a* concentrations within the samples (Box 4.5).

The chlorophyll-*a* concentration will provide an estimate of the standing crop or abundance of phytoplankton present in a water sample, but it will not provide any information on the composition of the phytoplankton present. To do this, you will need to identify and count each taxon (that is, each species or 'type') present using a microscope and a counting chamber. The data obtained by these means will provide an estimate of the number of cells per mL (cells. mL^{-1}) of each taxon and can be used to describe the composition of the entire phytoplankton community, the dominance of each taxon within that community, and changes in community abundance and composition over time. However, because different species of phytoplankton have cell sizes that differ greatly from each other, total cell counts are often unreliable for describing these changes. For example, a large cell count of a very small-sized algal species

BOX 4.5 EXTRACTION AND QUANTIFICATION OF CHLOROPHYLL

Chlorophyll-*a* is an indirect measure of phytoplankton standing stock (crop), and represents the mass of phytoplankton per unit volume or area of water and should be reported as micrograms per litre ($\mu g.L^{-1}$) or milligrams per cubic metre ($mg.m^{-3}$) or per square metre ($mg.m^{-2}$). Replicate water samples should be collected from the water column at pre-specified depths. The chlorophyll-*a* content is estimated in the laboratory using either the fluorescence or absorbance techniques described in Strickland and Parsons (1972). Water samples are filtered onto 25-mm diameter Whatman GF/F fiber (0.7 µm nominal pore size) or equivalent with a gentle vacuum (of less than 100 mm Hg). The actual sample volume can range from 100 mL to 4 L, as long as you can see that the filter paper is distinctively green. You should work in a shaded room because, in this state, chlorophyll can degrade in bright light. The sample can be wrapped in foil and frozen for up to 3 months for later analysis. The filter paper is extracted into 90% acetone and the light absorbance at particular wavelengths is recorded in a spectrophotometer. Alternatively, the natural fluorescence of the extracted chlorophyll can be determined – this is a more sensitive method.

may be replaced over time by a smaller number of cells of a much larger sized species. Using just the cell count data, you may deduce that algal presence has decreased, whereas, in fact, algal biomass may have increased. It may therefore be important, depending on the objectives of your study, to also determine the biomass present of each algal taxon identified and counted within the sample. Biomass is usually initially calculated as a biovolume ($mm^3.L^{-1}$), which is converted to biomass by assuming that algal cells have a density similar to that of water (therefore a biovolume of 1 $mm^3.L^{-1}$ equals a biomass of 1 $mg.L^{-1}$). Most correctly, biovolume estimates should be done by:

1. measuring the size of the cells of each species
2. converting this to an average cell volume for this species using standard geometric formula best representing the shape of the cell (Hillebrand *et al.* 1999)
3. multiplying the cell count by this average cell volume to obtain a total volume for all of the cells for that species.

This is often very laborious as it needs to be repeated for each species present in the sample. Sometimes published tables of standard cell sizes for various species are used instead, if the error involved is considered acceptable in comparison with the costs of using actual measurements.

Samples are best preserved using Lugol's iodine solution for both freshwater and marine samples (although it may damage some of the small

flagellates). Some laboratories will not analyse samples preserved with substances such as formaldehyde, as these are carcinogenic and represent an occupational health and safety hazard. Samples collected from a dense algal bloom can be analysed directly, but they usually need to be concentrated prior to analysis. This is usually done using a 100 mL aliquot of the sample that has already been well mixed by shaking the sample bottle prior to sub-sampling. The aliquot is poured into a 100 mL measuring cylinder and left to stand for a minimum of 24 hours. If small nanoplankton are present, a longer sedimentation time may be necessary. The Lugol's iodine preservative helps the cells sink more rapidly. After the required sedimentation period, most of the phytoplankton cells will have settled to the bottom of the measuring cylinder. The top 90 mL can then be drawn off using a suction pipette, taking care not to disturb the algal cells at the bottom of the cylinder. This gives a 10× concentration.

The identification and counting of phytoplankton cells is something that takes much patience, practice and experience to do correctly. There are a number of taxonomic guides and keys that have been published to assist in the identification of both freshwater and marine algae (see Chapters 5 and 6).

There are a number of methods available for counting algal cells in samples. The easiest method is using a Sedgwick-Rafter cell. Other methods (such as a Lund cell or an inverted microscope) are useful providing they can be used with at least as good an accuracy and precision as counts using a Sedgwick-Rafter cell (see Hötzel and Croome 1999 for a description of these methods). The Sedgwick-Rafter cell is a four-sided counting chamber that is 50 mm long by 20 mm wide by 1 mm deep, giving a bottom area of 1000 mm^2, and an internal volume of 1 mL. They have a grid engraved on the bottom, with lines 1 mm apart. If correctly calibrated and filled, the volume of sample covering each grid square is 1 mm^3. Both glass and plastic versions are available, with the glass cells being better, but more expensive. The cells are used on the stage of a normal compound microscope – preferably one with binocular eyepieces. Counting is done at 100× magnification, with higher power being used to identify small sized algal cells. A very thin microscope cover slip (No. 1 thickness) is required to cover the cell.

Immediately before commencing a count, the phytoplankton cells in the bottom of the measuring cylinder are resuspended into the remaining 10 mL of sample left in the measuring cylinder by swirling, and a further sub-sample of approximately 1 mL of this collected with a Pasteur pipette. This is then decanted carefully into the counting chamber of the Sedgwick-Rafter cell. The cell is full once the cover slip, which should be placed obliquely over the cell prior to filling with one corner open, just begins

to float and can be rotated to completely cover the chamber. This avoids introducing air bubbles into the sample. The cell should not be overfilled. Once filled, the counting cell should be left to stand on the stage of the microscope for 15 minutes, to allow the algal cells to settle to the bottom. It is not necessary to count all the cells on the bottom of the Sedgwick-Rafter cell. However, a minimum of 30 grid squares should be counted. These should be selected randomly, as there is differential sedimentation of algal cells within the counting cell, with more algae sedimenting closer to the walls than in the centre ('edge effects'). Counting traverses across the width of the cell helps to overcome these edge effects and will cover 40 grid squares. A second requirement is that a sufficient number of algae are counted to provide a counting precision of ±30%. This involves counting at least 23 'units' for all of the most dominant algal taxa present. A 'unit' is either an algal cell, filament or colony, depending whether the species being counted is unicellular, filamentous or colonial. If counting 30 grid squares or two traverses does not yield a sufficient number of units (that is, more than 23), then additional grid squares or traverses will need to be counted. Record the number of grid squares counted as well as the number of algal units counted. If an algal unit lies across the line engraved in the base of the Sedgwick-Rafter cell to delineate a grid square, so that it falls within two squares, the simple rule is that if it lies on the right side of the grid square, include it in the count, but if it lies on the left side, exclude it. Similarly, if it falls across the top line of the square, include it, but exclude any algal units falling across the bottom line. Algal units are often smaller than the width of the lines engraved in the Sedgwick-Rafter cell, so the same applies for any algal units lying within the grid lines delineating a square.

The number of algal units present per mL within the actual water body is calculated as:

$$\text{No. of units/mL} = \frac{(\text{units counted} \times 1000\ \text{mm}^3)}{(\text{no. of grid squares counted} \times \text{concentration factor, which is typically 10})}$$

For filamentous and colonial algae, it is then necessary to convert the count in units.mL^{-1} to cells.mL^{-1}. Many green algae have a set number of cells per colony (for example 4, 8, 16, or 32), so, when this is known, it is easy to multiply the units by the cell number per colony to obtain cells.mL^{-1}. However, many other phytoplankton species, especially cyanobacteria, have a variable number of cells per filament or colony. In this instance, it is necessary to count the number of cells in 20 to 30 randomly selected filaments or colonies, and then obtain an average number of cells per colony from these counts.

Further problems arise when samples contain large-sized colonies or tangled aggregations of filaments containing thousands of cells, where it is impossible to count all the cells in each colony or aggregation. In these situations, it is necessary to estimate a portion of the colony or aggregation – say 5% or 10% of the total colony size – and count or estimate the number of cells within that portion. Remember that the colonies or aggregations are three dimensional, with cells overlying cells, and outside of the focal plane at which you are viewing the colony. Once you have an estimate of the number of cells in 5% or 10% of the colony, multiply this by 20 or by 10, respectively, to obtain an estimate of the total cells per colony.

When you do these estimates of average cell numbers per filament or colony to obtain a count in terms of cells.mL^{-1}, the errors can be quite large and are in addition to any statistical counting error. The need to make these estimates arises only during blooms and becomes acceptable because of immediate management needs. Methods to break up large colonies into smaller units to make counting easier (homogenisation, addition of chemicals or sonification) are often inadequate and may destroy a large proportion of the cells present.

4.7 SAMPLING METHODS FOR ZOOPLANKTON

4.7.1 Mesh size, extrusion and avoidance

Zooplankton is typically collected with a fine mesh net, but using buckets or dip nets around bright lights is also possible. The appropriateness of mesh size can be determined through the trade-off between the net avoidance of zooplankton and net extrusion of zooplankton. With towed plankton nets, the smallest mesh size will never sample all the zooplankton, because larger and better swimming zooplankton will sense the pressure wave in front of a small mesh net and dodge it (this is known as net avoidance). If you use larger mesh, then the smaller zooplankton will be extruded through the mesh. We must accept that our sample is a selective view of plankton, but it will be a consistent view. The standard UNESCO mesh size for sampling zooplankton is 200 μm mesh (Harris *et al.* 2000) (Figure 4.4), but we have found that a 100 μm mesh is useful in estuaries as small zooplankton respond to environmental variability more rapidly than larger zooplankton (see Sections 3.7 and 4.8, 4.9). Many larval fish biologists use 500 μm mesh, knowing full well that fish eggs and small, unidentifiable larvae will be extruded through the mesh. Ultimately, net size should be determined in accordance with the objectives of your study.

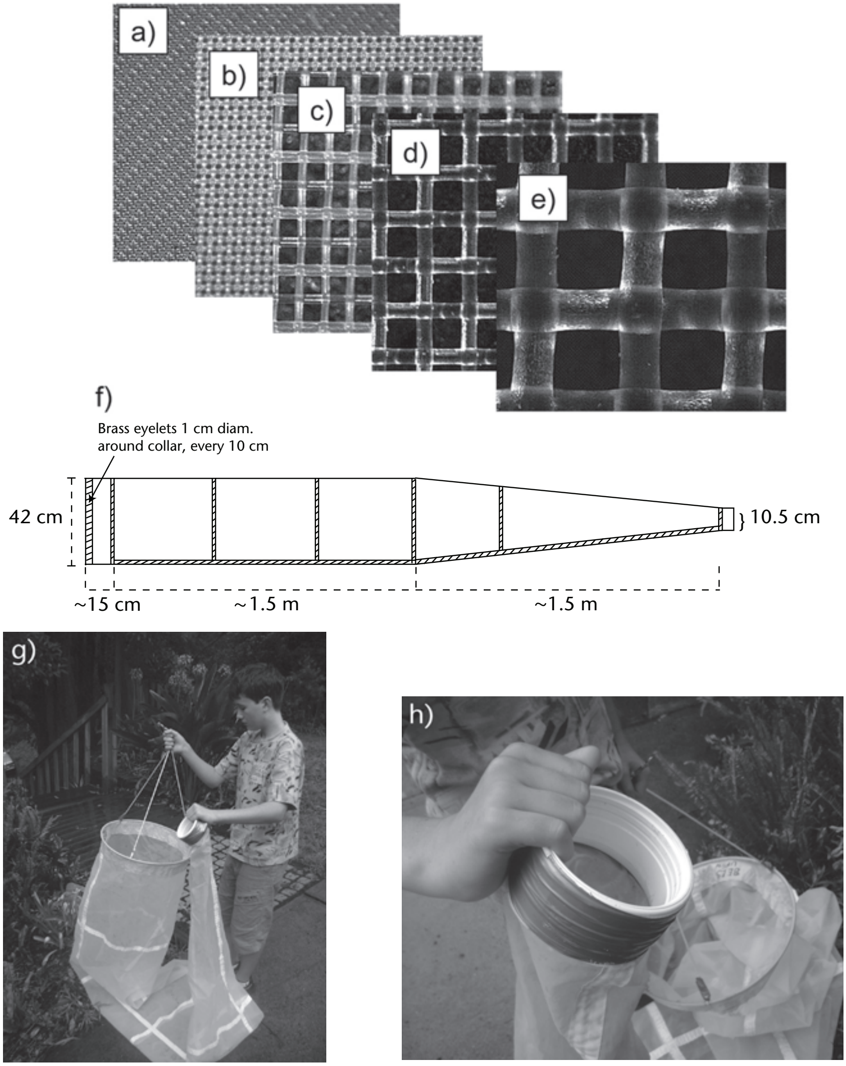


Figure 4.4 Plankton nybolt mesh at the same magnification. a) 15 µm, 10% free area, b) 48 µm, 31% free area, c) 150 µm, 51% free area, d) 250 µm, 44% free area, e) 500 µm, 39% free area, f) typical design for a 40 cm diameter ring net, g) mouth of a plankton net showing the bridle and attachments, h) the cod-end of the net, showing the thread made to suit the sampling jars.

Vertical hauls provide a depth-integrated plankton sample, and are useful for broad-scale spatial surveys of microplankton (less than 200 µm, small zooplankton and phytoplankton). The vessel must be stationary, and the net is either hauled up from a specified depth (an up-cast), or a heavy

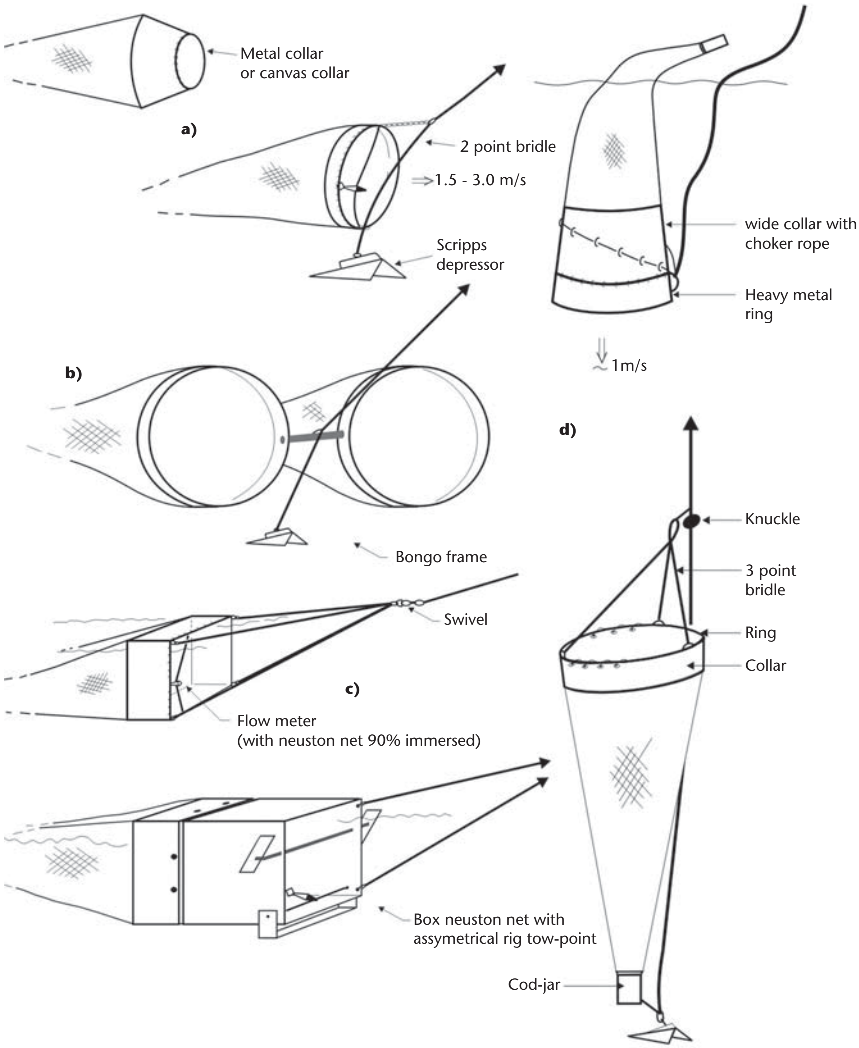


Figure 4.5 Types of plankton net, bridles and deployment. a) A standard plankton net configuration, with a two-point bridle and a depressor (note flow meter); a high speed plankton net with a sampling cone is illustrated, b) a bongo net sampler with no effects of the bridle, c) two neuston net samplers illustrating the robust four-point bridle and box neuston net sampler, d) gear for vertical hauls using a drop net or lift net (that is, down-cast and up-cast).

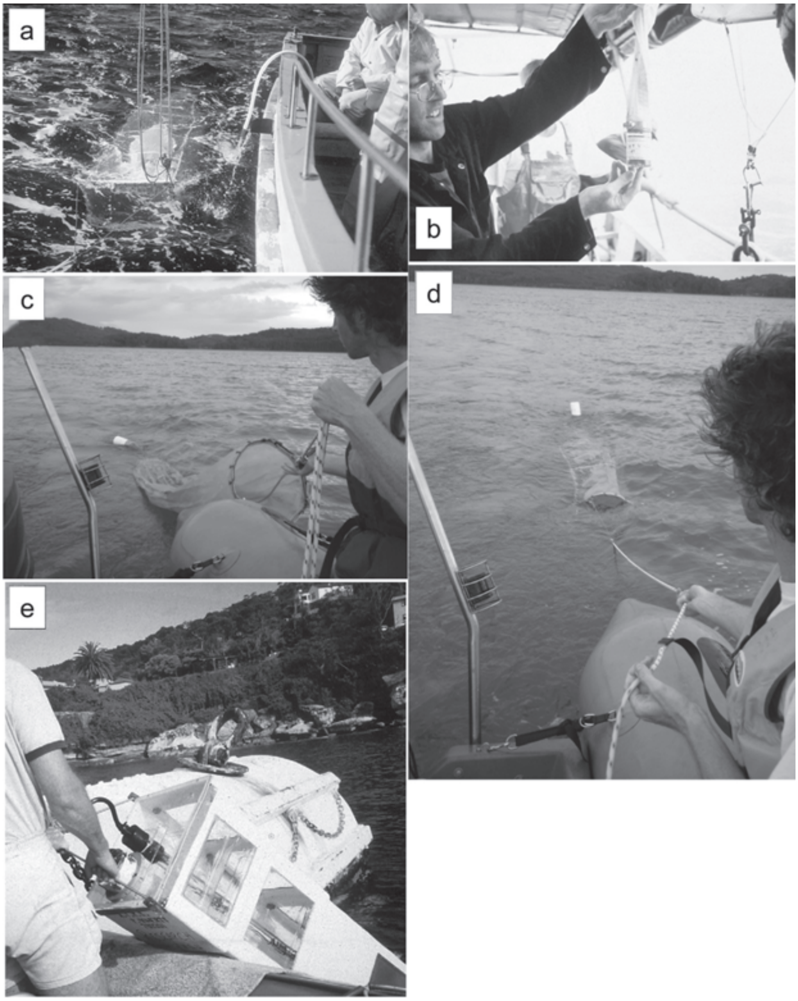


Figure 4.6 Some plankton collection gear. a) A square surface neuston net, b) a successful phytoplankton collection, c) deploying a ring net over the stern, d) beginning to tow the net in a circle to avoid sampling the propeller wash, e) retrieving a plankton light trap after a night's sampling.

metal ring (10–20 kg) carries the net down to a specified depth (a drop net or down-cast; see Figure 4.5d).

Zooplankton is collected horizontally by slowly towing the net at a constant speed – around 1–2 metres per second (Figure 4.6). Any faster will increase the extent of extrusion, and any slower may increase the incidence of avoidance. Nets may be fitted with a flow meter to determine the volume of water filtered (Figure 4.7), to then determine the number or biomass of zooplankton per cubic metre. For plankton sampling, you should be concerned with speed through the

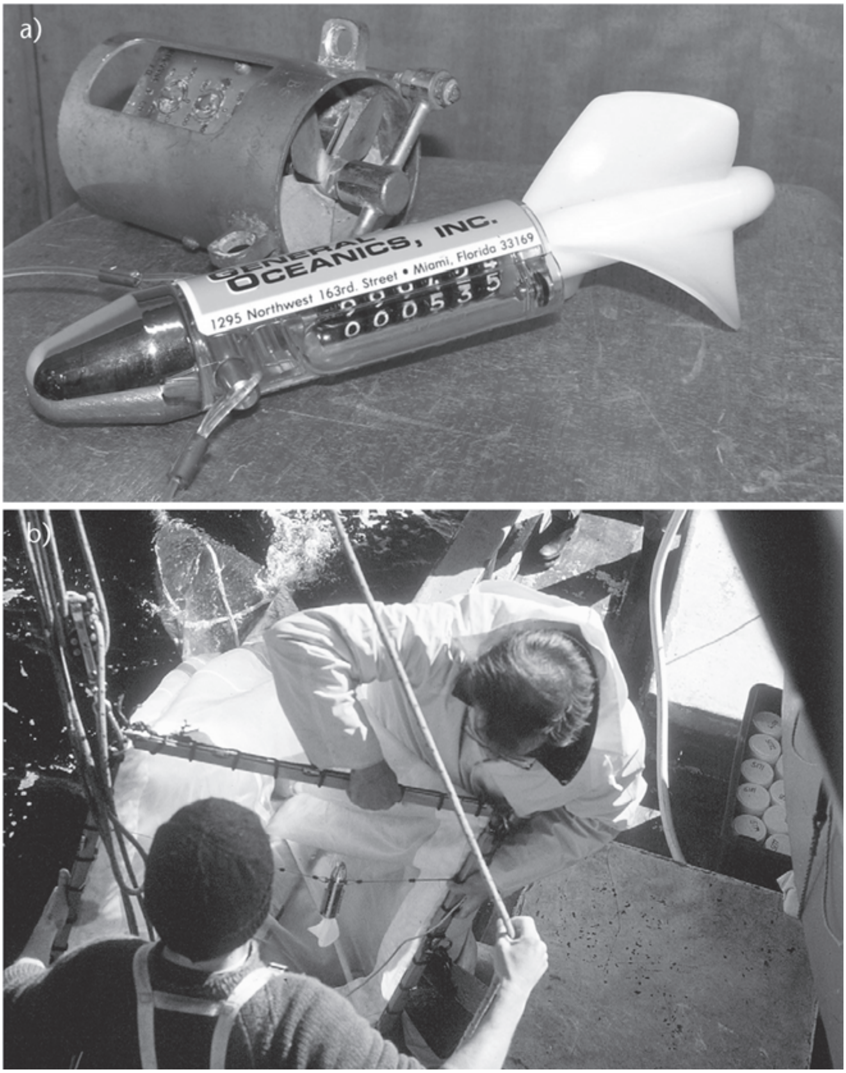


Figure 4.7 a) two types of flow meter and b) reading the flow meter before and after each tow (note that the flow meter is located to one side of the opening).

water, rather than speed over the sea floor. You should tow for a constant period of time (between 3 and 10 minutes, depending on mesh size and the amount of debris in the water) for a number of practical reasons. A constant sampling interval reduces potential sources of error such as sleepiness or sampling by a variety of personnel. Sometime flow meters break during the tow, or jam or become tangled with debris and, rather than dumping an un-metered sample, the volume filtered can be estimated with reasonable precision from the tow duration.

4.7.2 Net design and construction, and some typical plans

The frame of a circular net can be made with stainless steel round rod or, more cheaply, with stainless steel flat bar (Figure 4.4g). Stainless flat bar is stronger (with respect to the incident flow), cheaper per unit weight and easier to bend into a circle. A square frame can be welded from flat bar, but, again, stainless angle iron is stronger and cheaper per unit weight (although presents a slightly larger surface to the incident flow).

The bridle of the net is the harness, comprising ropes or strops that attach the mouth of the net to a tow rope. Three-point attachments on a ring net or four-point attachments on a square net are the most reliable, but may disturb plankton before they enters the net and may generate net avoidance. For a square neuston net the lower strops may be longer than the upper ones (Figure 4.5c). A circular net may have only a two-point bridle, causing less net avoidance (Figure 4.5a).

The net may be sewn by a local sail maker, in the shape of a cylinder and a cone leading to the cod-end (Figure 4.4f). The area of mesh must be nearly an order of magnitude greater than the mouth area of the net, so that there is a surplus of filtering surface area to cope with clogging and surface drag. A net is useless if there is a pressure wave in front of it resulting from

BOX 4.6 MANUFACTURE OF A SIMPLE RING NET (40 CM DIAMETER, 0.2 MM MESH)

For a typical 40-cm diameter ring net of 0.2 mm mesh (with a generous filtration surface area in case of minor clogging), make the net 42 cm diameter to comfortably fit over the ring (that is, radius or r = 0.21 m):

- Mouth area = $\pi r^2 \sim 3.14 \times (0.21)^2 = 0.139$ m^2
- Mesh porosity (that is, free area) ~ 50% >200 μm mesh; 30% (~ 100 μm)
- Mesh area = 10 × mouth area ÷ porosity (0.4) = 3.5 m^2
- Circumference of cylinder section = $2 \pi r = 1.32$
- Area of cylinder section 1.3 m long = 2.53 m^2
- Area of cone section 1.5 m long = ½ × circumference × length = 0.99 m^2

Adjust these dimensions to optimise the bolt of mesh. The collar of the net should be 20 cm wide and made of a strong polyester canvas, with brass eyelets 1 cm diameter every 10 cm around the circumference to lash onto the ring. Seams should be reinforced with polyester tape inside and out. The polyester canvas cod-end should be about 10.5 cm diameter to accept a PVC pipe coupler (held in place with a stainless-steel hose clamp), that has a thread on cut on the inside to match your plankton jars.

insufficient surface area. The shape and area of the mesh should be determined from the mouth area of the net, multiplied by a factor of 7 to 10, to account for the percentage free surface area (that is, the percentage area of hole, not the thread; see Figure 4.4a–e). This means that a typical 40 cm diameter net with 0.2 mm mesh is about 3 m long (Figure 4.4f, Box 4.6). Nets and towing devices can be designed to address specific questions. For example, neuston nets can be used to collect plankton from the surface or epibenthic sleds can be used to collect plankton just above the substrate. Talking to experts can help you to get specifications for these devices.

4.7.3 Simple plankton net (Figure 4.5a)

The bridle attachment may be a three-point or, with a weight such as a depressor, you may use a two-point attachment. Attach the tow rope to a solid mid-point near the keel (a strong seat or thwart), and ensure the tow rope does not press onto the motor (using a loop of twine). Samples are collected by slowly towing the net behind the boat and turning in a slight circle so that the net is not in your propeller wash (Figure 4.6c, d). Naturally, the inside of the boat's circle is the side with the net, and you have little manoeuvrability. Without any depressor or weight, the net will remain just beneath the surface at slow speeds. The drag on the boat is substantial, especially with larger nets, and care should be taken by securely tying the tow rope to the boat's strongest points. The railings of a small boat, or a bollard on the side is not the best tow point, because being on the side away from the motor thrust makes steering even more difficult. In boats more than 5 m long this is less of a problem. The tow rope may simply be tied around the thwarts or seat of an open boat, or even at the front anchor attachment and passing it over the transom near the stern. The net is best retrieved by turning off the engine and rapidly hauling it in hand over hand to prevent plankton from swimming out, or from dragging in the mud. If a winch is available, then it is best just to throttle back and haul the net up and into the flow. This is a simple, practical method, especially when working at night, but the boat's wash can still interfere with the net, potentially disturbing the zooplankton. Driving in a circle can be difficult in tidally flowing channels, and in the vicinity of fishing boats and pylons (Box 4.7).

The cod jar of a plankton net is a jar for draining and collecting the final sample (Figures 4.5d, 4.6b). It needs to easily screw into a PVC fitting (or 'coupling') that is ring-clamped to the cod-end of the net (Figure 4.4h). There are many individual designs for cod jars, but the simplest is to use one of your many sample containers – such as a 1 litre PVC jar. A workshop can turn your standard jar's thread into the PVC coupling. The jar will be

BOX 4.7 SAFETY NOTE

With all plankton net towing, the drag and pressure on the rope and boat is substantial – far more drag than simply dragging a similarly sized sheet perpendicularly through the water. A small boat towing a plankton net is not a common sight and many tourists and trawler fishermen will not expect you to be so slow and immobile. Sometimes they may come right at you out of curiosity, coming close to your tow rope. We generally avoid doing any plankton work during Christmas and school holidays specifically for this reason. Towing at night in estuaries near trawlers can be dangerous, especially as you have limited mobility and some trawlers may turn off their navigation lights.

brim full of plankton, so before unscrewing and spilling it, tip the excess water back out through the mesh, and splash water back up onto the mesh as a quick rinse-down.

After a day's sampling, your gear just needs to be soaked in fresh water and dried. With gentle tows plankton is easily rinsed off, but detritus jammed in the mesh must be dislodged with a good blast, and even a little detergent.

4.7.4 Other novel zooplankton samplers

Plankton pumps consist of a raft or boat supporting a power supply for a powerful centrifugal pump, which brings water from a particular depth to the surface and into a plankton net. The advantage of this system is that net avoidance is minimal, as the nozzle can be advanced through the water at the same rate as it is sucked in. Discrete depths and volumes can be accurately sampled. Some of the plankton may be damaged by the pump, but surprisingly little. The main disadvantage is the cost and noise of the pump.

Plankton purse seines are a relatively novel form of sampling gear for plankton. A sea anchor is cast out and the wall of net (260 µm mesh) paid out around a drift object (Kingsford and Battershill 1998). The sea anchor is then retrieved, before drawing the ends together, and pulling in the drawstring at the bottom. In the middle of the net is a cone shaped cod end, where the seaweed and plankton are eventually entrained. The net is useful for sampling moderately discrete volumes (about 50 m^3) at the surface, such as on plume fronts or around drift seaweed.

Some planktonic taxa are attracted to light, just like moths to a lamp. Sophisticated light traps have been built to turn on and off at intervals during the night (Figure 4.6e). They are most effective at sampling larger taxa in

Table 4.1. Summary of some common zooplankton sampling techniques.

Method/gear	Advantages	Disadvantages
SCUBA observations, with quadrat	• Animals in natural environment (jelly fish) • Natural densities • Behaviour • Small patches	• Macroscopic only >10 mm • Labour intensive • Diver avoidance? • Non-survey
Towed plankton nets, (e.g. bongo net, ring net; 20–1000 µm mesh)	• Quick, much replication • Neuston • Integrated sample of smaller patches, 20–10 000 m^3 • Okay in rough weather • Cheap (around $2K)	• Species/size selective • Extrusion/avoidance • Small patchiness? • Damage to animals • Vertical resolution?
Plankton pumps	• Small patch (<10 m^3) • Discrete depths • Known volume • Less gear avoidance	• Expense of big pump • Damage to animals?
Light traps	• Huge volumes sampled • Good condition, easy to sort • Catches material for experiments • Pelagic juvenile/ pre-settlement	• Selects those attracted by light • Volume filtered? • Attracts humans and ferries

an undamaged state, which would otherwise take many hours of pelagic trawl sampling (if at all). Apart from hauling them in and replacing batteries, light traps are easy to use, robust and simple. They work effectively during the new moon, and are selective for plankton attracted to light (just as any other piece of plankton gear is also selective). The volume that they 'filter' is unknown. Light traps are not particularly effective in NSW estuaries (compared with the Great Barrier Reef), except for some crustaceans, carangid larvae and herring or anchovy larvae.

4.8 PREPARATION AND QUANTIFYING ZOOPLANKTON (SUB-SAMPLING, S-TRAYS, PLANKTON WHEELS)

4.8.1 Observation of live plankton

Observing living zooplankton enables you to see how they use their swimming and feeding appendages and how they capture and consume food items. The colours and translucence of freshly caught zooplankton are amazing. You can capture live plankton around a bright light at night, or sample the contents of a gently towed plankton net. Live zooplankton cannot tolerate any trace of formalin or preservative or the heat of a lamp.

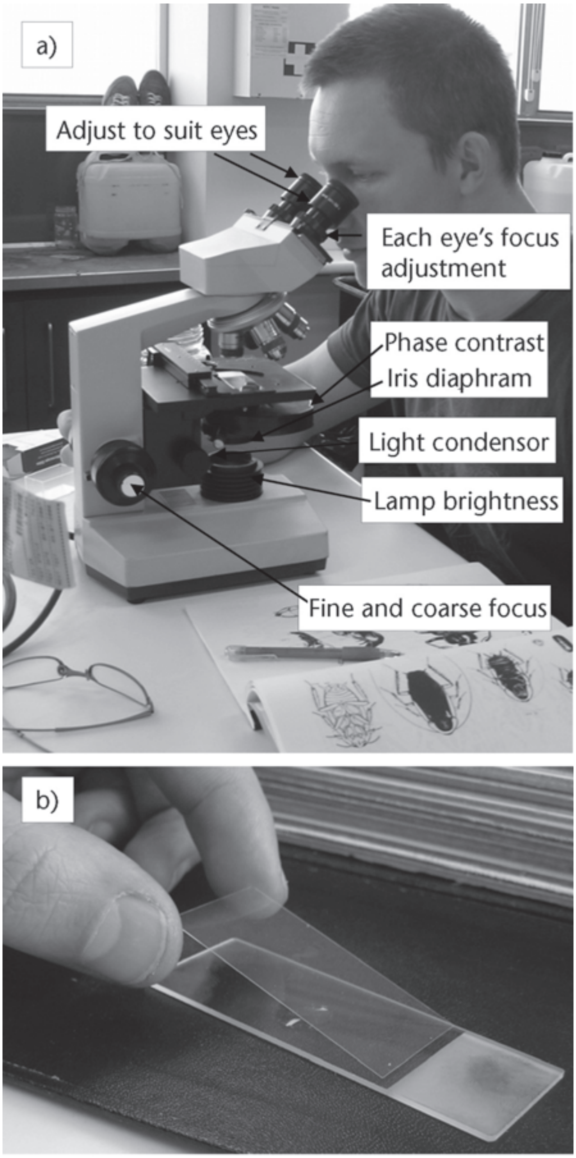


Figure 4.8 Compound microscope for phytoplankton. a) Viewing a plankton sample should be relaxing, without squinting or using only one eye. By adjusting your chair height you should have a straight back and neck. Adjust the eye-pieces to suit your own inter-ocular distance (see Figure 4.9b; by closing each eye separately you should have an unobstructed view); after adjusting the coarse and fine focus knobs for one eye, you may also need to twist one of the eye-piece's individual focus adjustments. b) Method for preparing a wet mount for a compound microscope.

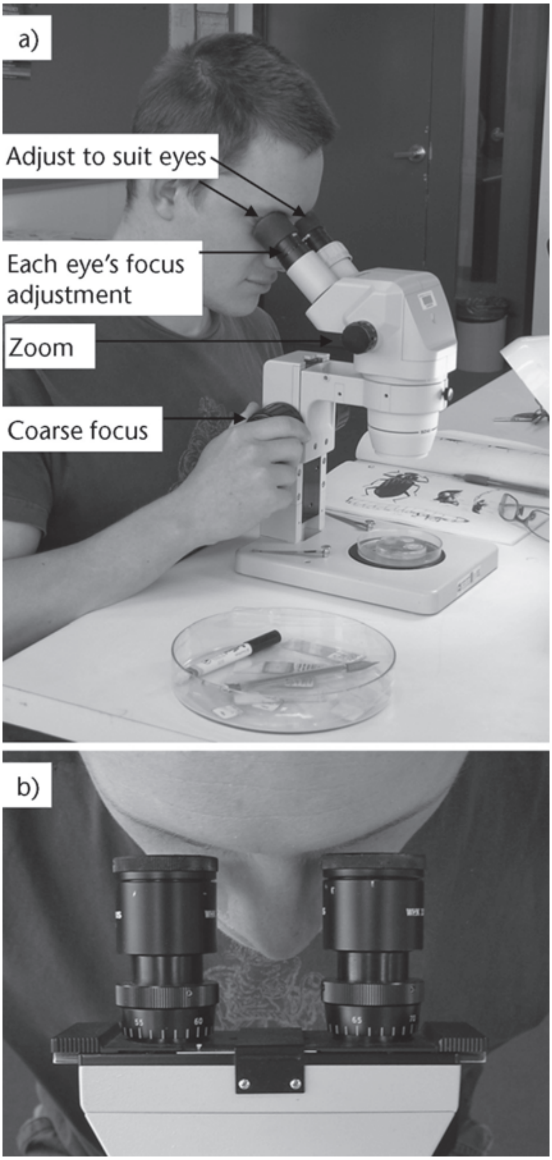


Figure 4.9 a) Dissecting microscope for zooplankton, b) ensuring the eye pieces are adjusted to suit your inter-ocular distance.

For large living zooplankton, use a wide-mouth pipette to place a small volume of the sample into a clean Petri dish. It is best to observe large copepods and cladocerans under the dissecting microscope at low magnifications (less than ×40), as then they remain focused in the larger depth of field and they are less able to swim out of the microscope's field of view (Figures 4.8, 4.9). An anaesthetic (for example, a few drops of $MgCl_2$ solution, soda water, clove oil or some ice) will slow the activity of larger zooplankton.

For small living zooplankton, use a pipette to place a small volume of the sample into a clean observation chamber, such as a counting chamber. A counting chamber can be made with two glass cover slips placed 3–10 mm apart, and a few drops of the sample placed between. Then gently place an intact cover slip over the sample, resting on the two beneath. The water will be held in the small chamber to prevent zooplankton specimens from being squashed between the slide and the cover slip. If an inverted microscope is available, you may observe living zooplankton held in a small volume of water on the glass slide without placing a cover slip.

4.8.2 Sorting a zooplankton sample

The laboratory analysis should also be guided by what the investigator requires, and by the budget. Sorting and identifying zooplankton to a reasonable degree of accuracy is arduous and may take 1–4 hours per sample. Could your question be resolved by zooplankton biomass or by identifying to the level of phylum, family or genus? Perhaps only the Crustacea – the greatest phytoplankton consumers – need to be identified. Or is a size analysis sufficient? Will you sort two or three subsamples, or do you plan to sort the entire sample for fish larvae only? You should prepare a sorting data sheet to complement the field data sheet (Figures 4.10, 4.11).

The sample should first be rinsed in a sieve (of the same or smaller mesh of the net) to remove formaldehyde solution, and to remove/rinse grass and sticks. Rinsing with cold fresh water is perfectly adequate for preserved plankton. Gelatinous zooplankton should be counted and removed at this stage, and recorded on your field data sheet (Figure 4.10). Then carefully rinse the plankton from the sieve into a beaker or a 100 mL volumetric cylinder (if necessary make up the volume to 100 mL). With bulky samples, especially with detritus, a 200 or 500 mL cylinder may be necessary. Allow a uniform time period for the plankton to settle (about 1 hour), and read off the approximate displacement volume (that is, the approximate volume in

FIELD DATA SHEET

Crew: ______________________________ **Sample ID code:**________________________
Date:_______________________________ **Time:** _________________________________
Location/GPS: _______________________
Station:_____________________________ **Depth:** ________________________________

\- — -

Weather:
Wind speed/direction: ________________ Waves/tide/current: _____________________
Air temp:______________________________ % cloud: ______________________________
Moon phase: ________________________

\- — -

Water @ start:
Temperature/Salinity: _________________ °C_______________ Secchi depth: ________
pH: _______________________ DO: _____________
Comments:

Sampling gear:______________
a) Sample #: _______________ Time: _____________ Flowmeter: _________________
b) Sample #: _______________ Time: _____________ Flowmeter: _________________
c) Sample #: _______________ Time: _____________ Flowmeter: _________________
d) Sample #: _______________ Time: _____________ Flowmeter: _________________
Comments:

\- — -

Water @ end:
Temperature/Salinity: _________________ °C _______________ Secchi depth: ________
pH: _______________________ DO: _____________
Comments:

Figure 4.10 A typical plankton field sampling data sheet.

millilitres of zooplankton – normally zooplankton is added to the water). Detritus tends to sink slower than zooplankton, while any sand grains will sink faster, enabling you to estimate the actual zooplankton biomass.

After you have recorded the displacement volume, thoroughly mix the zooplankton in the volumetric cylinder, and while still swirling remove an accurate 2 or 4 mL sub-sample with a pipette (with the fine tip cut off, Figure 4.12). Thus you have removed 2 or 4% of the total sample, such that

LABORATORY DATA SHEET

LOCATION: ______________________**STATION #:** __________________________

Sorter's name: _____________________**Date:**_________________________________

Sample #: _________________________**Location:** ____________________________

Gear and mesh: ___________________**Tow duration/speed:** ___________________

	Sample#	Comments: (sub-sample?)	Sample#	Comments: (sub-sample?)
copepods calanoid cyclopoid harpacticoid				
bivalved crustaceans ostracod cladoceran				
crab larvae				
amphipod isopod				
nauplii				
elongate crust. krill mysids penaeids *Jaxea*				
polychaetes				
chaetognaths				
pelagic snails				
bivalve molluscs				
cnidaria *Obelia*				
larvaceans				
salps				
other gelatinous				
fish eggs				
fish larvae				
large jellies, ctenos, algae?				

Figure 4.11 Possible laboratory data sheet.

you multiply your counts by 50 or 25 to get an estimate of total number. The volume of the sub-sample should be determined by the density of zooplankton and the time it takes to sort. It is better to take two or three 1 mL sub-samples, rather than one 3 mL sub-sample, as the variance due to sub-sampling error can be incorporated into your analysis. (Remember to account for the fact that the second and third sub-samples are not the

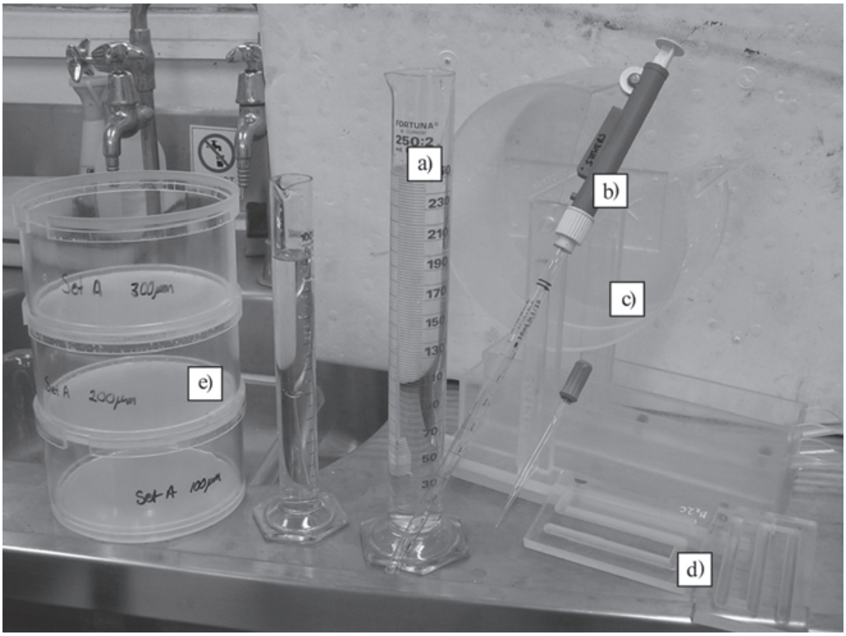


Figure 4.12 Typical plankton sorter's equipment showing a) volumetric cylinders for determining settlement volume, b) a blunt-ended pipette with a deliverer to take a quantitative sub-sample from the well-mixed sample thoroughly suspended in 100 mL or 250 mL of clean tap water (a non-quantitative Pasteur pipette is included), c) a plankton splitter for dividing a plankton sample into half, thence a quarter, and eighth, and so on, d) an 'S' tray for counting samples, e) a series of stacked home-made sieves to size-sort plankton with 300, 200 and 100 µm mesh.

same proportion of the total as the first – although the error introduced by ignoring it is minor compared with other factors).

The sub-sample is best sorted and identified in a Bogarov tray or an S-tray (a perspex square with a 1 cm deep trough milled into it, Figure 4.12d), or in a plankton ring (a perspex ring that can be rotated under the microscope). Your laboratory data sheet should be beside you (Figure 4.11). Some fine probes are useful in turning individuals to identify them (Box 4.8). Your counts could be dictated onto tape if you wish, and thence transferred onto the spreadsheet, where you can insert the necessary formulae to correct for sub-sampling and the total volume filtered (below). The remaining sample may be scanned for any large or interesting plankton, before storing it in 2% formaldehyde in fresh water.

BOX 4.8 FABRICATION OF TUNGSTEN WIRE PROBES

Tungsten wire probes are very fine and firm needles for sorting tiny plankton. The wire may be sharpened by electrolysis (Conrad *et al.* 1993). A mild electric current is passed between a 3 cm length of wire and an electrode immersed in a 1.0 M solution of sodium hydroxide (20 g of NaOH pellets in a litre of water). With an electric current, the tungsten tip is delicately dissolved only as it is dipped into the solution. You will need a source of magnification to observe and regulate the sharpening. The rate of electrolysis is proportional to the surface area of the wire, the amount of current and the concentration of NaOH. A microscope's AC light source can provide a variable current, with alligator clip leads. Once the wire is sharpened, the other end may be glued or fixed onto handles. You need to exercise usual care with all aspects of the process, including handling the caustic solution, using the electric current and handling the sharp needles.

4.8.3 Fixation and preservation of plankton

A fixative, such as formaldehyde, chemically treats the tissues: stopping biochemical activity and increases the mechanical strength. A preservative, such as alcohol or salt, is a natural compound that reduces or stops decomposition without chemically fixing the tissue. Samples preserved in alcohol may shrink or become distorted more than in formaldehyde, but are safer and more pleasant to study, and are suitable for DNA analysis. Therefore the type and amount of fixative/preservative used should be determined by the sampling objective and the size of the samples being collected (Table 4.2). If preservatives are not available, the samples should be kept cold – either stored in a refrigerator or stored in a portable icebox. Under these conditions, however, the samples are only viable for a period of 1–2 days.

Table 4.2. List of possible plankton fixatives.

Phytoplankton fixative	30% methylated spirits 5% glutaraldehyde Lugol's solution* Tincture of iodine* Acid Lugol's 2% formaldehyde
Microzooplankton fixative	2% formaldehyde
Macrozooplankton fixative	5% buffered formaldehyde (37% formaldehyde with sodium tetraborate or hexamine).Rinse and transfer to 70% alcohol for long term preservation.

* NB: When adding tincture of iodine or Lugol's to the sample, do so 'drop by drop' until the sample turns a dark tea colour.

Formaldehyde is usually made from the oxidation of methanol, using silver or copper as a catalyst. The concentration provided by the manufacturer is typically a 40% solution, with a trace of methanol to reduce polymerisation to paraformaldehyde (a white precipitate – which may be cleared by warming or with a few pellets of sodium hydroxide). This concentrated solution is pungent and carcinogenic (Box 4.9). Sometimes it is hard to tell (during arduous or sleepless field conditions) if formaldehyde has been added to the plankton sample. A few drops of a stain such as eosin in your 40% stock solution is a useful indicator.

You only need a very dilute solution to preserve plankton, and such dilutions are sometimes termed 'formal', 'formol' or 'formalin', but these are imprecise and are discouraged. A 4% solution of formaldehyde (such as for preserving fish or macrozooplankton) is made up from 10 mL of the 40% commercial or concentrated grade and 90 mL of sea water or fresh water. This solution should be referred to as '4% formaldehyde', not as '10% formalin' (as this author and others have sometimes used). Similarly, for preserving zooplankton, a 1 or 2% formaldehyde solution is used, which is made from 25 or 50 mL of 40% concentrated formaldehyde and made up to 1 litre (Steedman 1976). This may also be buffered with a few marble chips. In a tightly sealed jar, this solution is stable for decades if stored in a cool and dark location. Do not squeeze too much plankton into a sample jar – the volume of plankton to solution should be about 1:9 (Steedman 1976).

Before sorting such a sample, it is best to gently rinse off the formaldehyde solution thoroughly in fresh water, and transfer to 70% alcohol as a preservative. Alcohol is a good long-term preservative, but it does not fix animal protein histologically. Formaldehyde solution may be buffered with sodium carbonate ($NaCO_3$, purchased cheaply in bulk as 'soda ash') as a 5% formaldehyde solution becomes slightly acidic, which dissolves calcium carbonate, including larval fish otoliths (which are used to determine age

BOX 4.9 OCCUPATIONAL HEALTH AND SAFETY

Note that fixatives and preservatives are poisonous and some are probably carcinogenic. Adequate care should be taken at all times. Examination of live, non-preserved samples is best. Otherwise all samples should be preserved immediately, or should be placed in dark cool containers (eskies or fridges) to ensure that no further primary production or grazing can take place. Consult with the personnel at any identification laboratories regarding the method they require and remember that some researchers also like to get a separate live sample that can aid them with the identification of small flagellates and ciliates.

and daily growth). After a few weeks, buffered larvae suffer bleaching of their black spots (melanophores). It is best to transfer fish larvae to 95% alcohol within weeks of capture (70% alcohol is also slightly acidic).

4.9 AUTOMATED METHODS FOR ZOOPLANKTON SAMPLING: EXAMPLES OF SIZE STRUCTURE

Recent image analysis and video analysis instruments can make some automated identification of plankton (such as 'Flowcam' or 'Video Plankton Recorder'). One need only imagine the different orientations of a translucent copepod – along with all the many copepod naupliar and copepodite stages of each species – to realise the difficulty of such a process. Identifying plankton to genus and species is beyond most budgets, unless there are specific algae (toxic) or larval crustaceans and fish (commercial). Therefore some plankton ecologists resort to classifying plankton quickly and cheaply by size. Small particles are very abundant, while large particles are exceedingly rare – a general phenomenon known as the biomass size spectrum. Size is correlated with many ecological rates (Section 3.7), and the size frequency distribution can, for example, indicate the overall productivity in response to nutrients. A size-based analysis is based on the assumption that biomass is transferred from smaller to larger sizes by predation. Therefore some larval prawns are equivalent, in terms of size, to most copepods.

One limitation of size analysis is that debris, which may be abundant in estuarine and coastal waters, may be counted along with the zooplankton. Also, knowledge of certain key species or indicator species will not be known unless some calibration samples are inspected. At high zooplankton concentrations the instrument will suffer from two simultaneous particles being counted as one – which is termed 'co-incidence'.

There are a number of size-based plankton counters, particularly for small particles such as bacteria and phytoplankton (such as the Coulter counter, flow cytometry and HIAC particle counters), but these are specialised instruments operating from a laboratory.

One of the major field instruments for counting and sizing zooplankton in the 0.3–3 mm size range is the Optical Plankton Counter. The instrument counts and sizes plankton as it flows through a small sampling tunnel and interrupts a thin red light. The decrease in light intensity received by the sensor is recorded as a particle and converted to an area and thus an equivalent spherical diameter. Size is converted to biomass using the volume of a sphere and assuming a density of water. The sensor must receive a constant illumination, such that in turbid water, the light output must be increased,

which is recorded as light attenuance (so one records counts, sizes and turbidity). The size categories can then be cross referenced with some typical taxa.

A cheaper semi-automated method is to count and measure the individual areas of your preserved plankton sample with image analysis – using a CCD camera mounted onto a dissecting microscope. There are a number of public domain image analysis packages. The plankton sample may be stained with any histological dye (such as lactophenol blue or chlorazol black), and a sub-sample placed into a Petri dish. A number of images of different areas of the sample are recorded, which are then contrasted and the resultant blobs

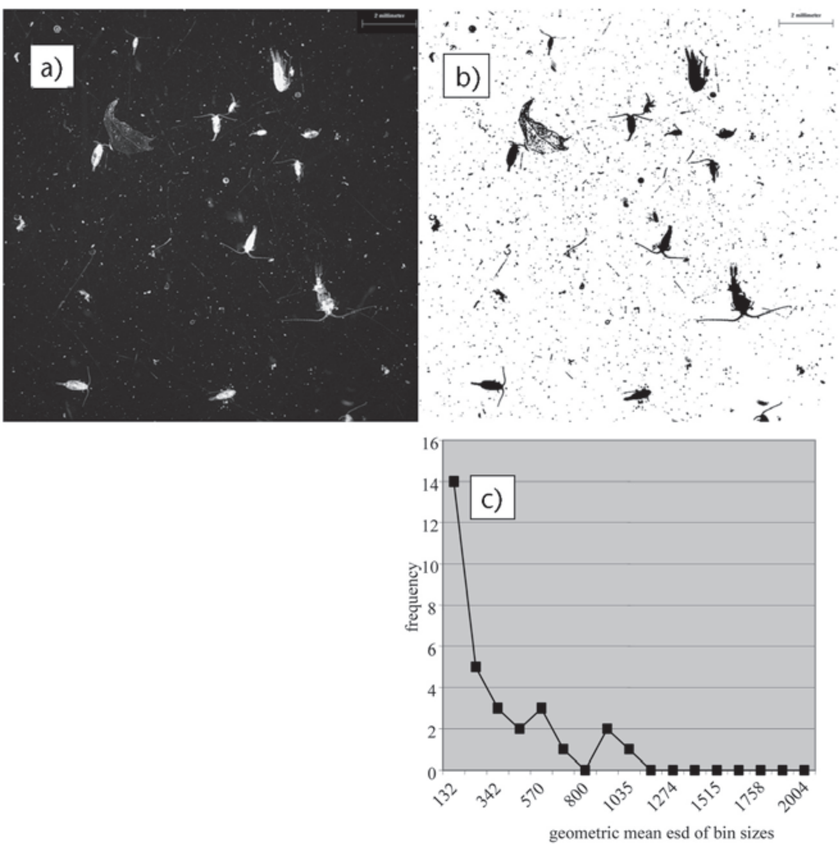


Figure 4.13 Three steps to produce a zooplankton biomass size distribution from a) an image of zooplankton. The image is adjusted to a standard level b), and the areas of the blobs are determined and converted to equivalent spherical diameter (Area=πr^2) and displayed as a frequency histogram c) as numbers of bugs per mL of concentrated sample.

on the screen are counted and sized. A critical stage in this analysis is to optimise the appropriate sub-sample and dilutions, to prevent too much co-incidence and yet to have reasonable number of counts per grab. The actual particle concentration of each size category is determined by multiplying up from the sub-sample volume and the actual volume filtered.

The intercept and slope (negative) of the log-based biomass size distribution is a useful parameter of the plankton population dynamics (see Section 3.7). For analysis, any particular size intervals may be used, beginning at around three times larger than the net's mesh size. This is because many zooplankton species are shaped like an oblate spheroid, so that the smallest equivalent spherical diameter fully sampled by a certain mesh size is around three fold larger. Any size classes – linear or logarithmic – may be used, providing one converts the biomass to 'normalised biomass size spectrum' (NBSS, dividing the biomass concentration ($mg.m^{-3}$) of each size class by the biomass size interval). For smaller estuarine zooplankton with 100 µm mesh, we use 24 size limits set at 18^2 to 40^2 (that is, size intervals at 324, 361 etc to 1600 µm equivalent spherical diameter).

4.10 METHODS: ANALYSIS, QUALITY CONTROL AND PRESENTATION

Your data should be standardised as numbers per unit volume filtered – as indicated by the flow meter (litres, m^3, 10 m^3, 100 m^3, 1000 m^3 and so on). Generally the standard unit of volume should be similar to the actual volume of water filtered. For example, many of our neuston tows filter 200–300 m^3, so we would report our results as numbers per 100 m^3. Some surveys quote numbers per unit area, by multiplying the concentration by the station depth, thus estimating the numbers of larvae per unit area of ocean (see Box 4.10).

A flow meter to estimate the number of zooplankton per cubic metre of water filtered (m^3) is necessary for nearly all plankton work. The mouth area of the net (πr^2) times the velocity will provide the maximum volume filtered (spillage around the mouth of the net is inevitable, depending on tow speed and clogging). This volume-filtered may be visualised as a column of water: the diameter of the net and the length of the tow.

There are two basic types – the General Oceanics (GO) or the barrel type Tsurumi-Seiki Co. (TSK) or Rigosha & Co. (Figure 4.7). The GO flow meters have a 6 digit number that increments by 10 for every revolution, and the number must be recorded at the beginning and end of each tow. The difference is used to calculate the volume filtered.

BOX 4.10 CALCULATING COPEPODS PER CUBIC METRE

1. Calculate the distance through the water for the flow meter
2. Sampled volume (V) for a 40 cm diameter net towed at 1 m per second for 5 minutes is the volume of a cylinder, which is the mouth area times the distance towed:

$$V = \pi \times (0.4/2)^2 \times 1\ \text{ms}^{-1} \times 300\ \text{s} = 37.7\ \text{m}^3$$

3. If the average number of copepods in your 2% samples = 45, then the concentration of copepods per cubic metre of lake water (C) is:

$$C = (45 \times 100/2)/37.7 = 59.7\ \text{copepods.m}^{-3}$$

Numbers per m^3 or m^2? Survey results of phytoplankton or larval fish may be reported in numbers m^{-3} or numbers m^{-2}. The former statistic is a concentration, while the latter is an overall abundance throughout the water column for that station. The areal abundance is calculated by multiplying the concentration by the bathymetric depth of the station (provided that you made an oblique or vertical haul, sampling the whole water column). In estuaries where you usually sample a fixed depth (surface or at 3 m), and where bathymetric depths can vary substantially, it is best to use a concentration.

The formulae for calculation of volume (from their manual) are as follows:

1. Distance (m) = (difference × Rotor Constant)/999 999
2. Speed (cm s^{-1}) = (Distance (m) × 100)/duration of tow (s)
3. Volume (m^3) = (3.14 × r^2) × distance (m)

Putting formula (3) into a spreadsheet is simple, and does not require you to time the duration of the tow (but a standard 5 or 10 minute tow is a good safety standard, if the flow meter jams). The rotor constant for a new standard rotor is 26 873 and this should be checked by attaching it to a rod and walking it briskly along a 50 m swimming pool. The axle of the propeller is delicate and prone to being bent, corrosion can affect the internal mechanism if the meter is not flushed and dried after use and seaweed may jam the rotor during a particular tow (and hence the importance of a standard 5 or 10 minute tow).

Sampling plankton entails the use of many vials and jars, which when sampled in various impact and control sites requires a good system to be in place to ensure that data are not mixed up. Label your jars with a unique number, which should travel through to the field data sheet

(Figure 4.10), spread sheet and analysis. To ensure compatibility and accuracy, also record:

- water collecting device and dimensions
- depth of water samples (m) and their volume (mL)
- number of stations sampled and number of samples collected
- analyses performed and laboratory methods used
- water temperature, nutrients, light and salinity
- identity of species using field guide
- preservative used and volumes of sub-samples

BOX 4.11 SAFETY AND CARE

Legislation. In many places, you may be required to obtain a permit to collect samples from a government authority. Make sure you have considered this before going into the field. It is useful to let local authorities know about your activities as community members may be alarmed if they see you sampling, particularly if you are using a fine mesh net.

Safety procedures while plankton sampling

- Use common sense.
- Do not use a boat without a boat drivers license.
- Ensure your boat and engine are properly maintained.
- Always notify someone of your proposed boating activities before leaving and notify them again when you return. Provide them with an estimated time of return and let them know the approximate areas you will be sampling.
- Look at a map of the site and select appropriate boat ramps.
- Use plenty of sunscreen (water reflects back additional radiation).
- Take maps, mobile phones and/or VHF radio.
- Ensure that you have sufficient fuel.
- Entrance bars can cause extremely dangerous conditions – never leave the mouth of an estuary to enter the ocean unless you are with an experienced boat handler and in a suitable boat.
- Depending on the boat you are using, keep checking the weather as swells can develop rapidly in some systems and can cause problems with small boats.
- Carry appropriate equipment, such as life jackets, oars, rope, anchor, torch, bucket and water.
- Don't overload your boat.

- station/transect/grid location
- date, time of day sampling conducted.

The individuals/teams collecting data should undergo training and should be provided with a comprehensive list of actions and requirements while sampling (Box 4.11). This ensures consistency among, and between, teams. Field notes and data sheets are essential and a chain of custody should be in place through which the sample can be tracked back to the collection stage. Information about detection limits, methods and standards used should be provided and should be consistent with the objectives and hypotheses of the management plan/ monitoring program. With certain types of variables it is often useful to conduct inter-laboratory comparisons.

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Chapter 5

Freshwater phytoplankton: diversity and biology

Lee Bowling

5.1 IDENTIFYING FRESHWATER PHYTOPLANKTON

The group commonly referred to as 'algae' constitute a large and very diverse assemblage of organisms. Up to 15 different groups or 'divisions' are recognised, depending on the system of classification used. Although there may be some superficial similarities between these divisions, they can differ greatly from each other, especially in regards to their pigment arrays and their cellular ultrastructure. The evolutionary relationships between many of these divisions are thus obscure.

A number of these algal divisions occur predominantly in fresh water and have only a few marine representatives, while others are well represented in both the marine and freshwater environments, albeit by different genera. Additionally, even though some divisions may be present in fresh water, they do not form part of the phytoplankton communities, but instead grow attached to a substrate – examples include stonewarts (Charophyta), and freshwater species of red algae (Rhodophyta).

Some phytoplankton are extremely small, with cells of less than 1 µm in diameter. Even the larger freshwater phytoplankton cells may be only up to 500 µm in their maximum dimension. The majority, however, fall within the

nanoplankton and microplankton size ranges, although the abundance, role and importance of freshwater picoplankton algae may be often overlooked because of their small size. Some colonial and filamentous phytoplankton species may form aggregations up to 2 mm in diameter, and be visible to the naked eye.

Today there is an increasing reliance on DNA-based molecular techniques for identifying phytoplankton species, especially for toxigenic species where reliable identification is necessary for the protection of public health. However, a range of morphological features have traditionally been used in the microscopic identification of freshwater phytoplankton, including:

- the size, shape and colour of cells
- the arrangement of cells (single, filamentous, colonial)
- the type of cell wall
- the presence, absence and positioning of flagella and other distinguishing organelles and specialised cells.

Many of these features are distinctive to each division of algae. This chapter presents summary descriptions of the main divisions of phytoplankton that occur in freshwaters, to illustrate the diversity found within these organisms in this environment (see Chapter 6 for the marine phytoplankton). Far more detailed descriptions and references to original research can be found in specialist textbooks on algae (for example, Bold and Wynne 1986; South and Whittick 1987; Van Den Hoek *et al.* 1995; Lee 1999). Details of the ecology and reproductive strategies of many of the different divisions of freshwater phytoplankton may be found in Sandgren (1988a).

5.2 CYANOBACTERIA (BLUE-GREEN ALGAE)

The most striking example of the great variation and differences between phytoplankton comes when the cyanobacteria – or 'blue-green algae' – are compared with all the other algae (Box 5.1, Box 5.2). Cyanobacteria belong to the Kingdom Eubacteria, which, together with the Archebacteria, makes up the Prokaryota. Prokaryotes are organisms whose cells possess little internal organisation and lack organelles (such as a nucleus or mitochondria), which characterise the eukaryotes.

All other types of algae (and indeed all other cells) are eukaryotic organisms, in which there is separation of different cellular functions into distinct membrane-bound organelles within the cell. These types of algae have a closer affinity to the higher plants than to the bacteria. Cyanobacteria also

BOX 5.1 CYANOBACTERIA AND OTHER PHOTOSYNTHETIC BACTERIA

As well as cyanobacteria, red and purple photosynthetic bacteria also occur in some lakes and ponds. However, there are marked differences between the two. Cyanobacteria have in common with eukaryotic algae the presence of the pigment chlorophyll-*a*, which is used to trap light energy for photosynthesis. The biochemical pathway for photosynthesis in cyanobacteria is exactly the same as that in other algae and the higher plants – where carbon dioxide and water are used as the basic ingredients to manufacture carbohydrates, and oxygen is liberated in the process. In addition to chlorophyll-*a*, cyanobacteria also possess the accessory light-trapping pigments phycocyanin and phycoerythrin, which are blue and red coloured, respectively, and give the cyanobacteria their distinctive blue-green colouration. In contrast, the photosynthetic bacteria possess pigments other than chlorophyll-*a* (they have instead bacteriochlorophylls), are obligate anaerobes (must live in environments devoid of oxygen), and they do not release oxygen as a result of their photosynthetic processes (unlike cyanobacteria).

BOX 5.2 BUOYANCY REGULATION IN CYANOBACTERIA

Although the cells of cyanobacteria do not possess any internal structure or flagella, many planktonic species, but not all, do contain gas vesicles, which can form larger aggregations known as gas vacuoles, and which may be observable under light microscopy as black speckles within the cell. Gas vesicle production provides the cells with positive buoyancy, enabling them to float up through the water column towards the surface to obtain additional light for photosynthesis. Photosynthesis leads to the accumulation of denser carbohydrate metabolites that increase ballast, and also increases turgor pressure within the cells that will collapse the gas vesicles. These mechanisms lead to the cells sinking again (Oliver 1994). Using their buoyancy regulation mechanisms, cyanobacteria can actively migrate up and down the water column – usually rising towards the surface in the early morning, and sinking during the afternoon. It has been proposed that sinking into deeper waters may allow the cells to obtain additional soluble nutrients that can accumulate at depth. However, Bormans *et al.* (1999) consider that vertical migrations only occur within the surface mixed layer, and do not extend down into these deeper nutrient-enhanced waters.

have other features that they share with bacteria. Under certain conditions – especially when there are low concentrations of nitrogenous nutrients present in the water column – many of them can fix atmospheric nitrogen into organic nitrogen (Box 5.3). This is a feature that they share with some other bacteria, such as those that live in the roots of leguminous plants

BOX 5.3 HETEROCYTES AND AKINETES

Cyanobacteria within the Order Nostocales can produce two types of specialised cells that are not found in the other two orders discussed here. The first are the heterocytes, where nitrogen fixation takes place. Heterocytes usually have thickened walls to exclude oxygen, the presence of which prevents nitrogen fixation. However, heterocytes may not be present if there is plenty of bioavailable nitrogen present within the water column, because fixation is therefore not necessary. The other type of specialised cells – called akinetes – are resting cells or spores produced from vegetative cells. These also develop thick walls, have concentrated food reserves and sink and remain in the bottom sediments until environmental conditions suited to a renewed bloom reoccur. The akinetes then germinate and commence a new bloom. Akinetes also may not always be present, but frequently develop when environmental conditions become unfavourable for the continuation of an existing bloom. The location of the heterocytes and akinetes within the filament are some of the morphological features used to distinguish different genera and species within the Nostocales.

(such as lupins and clover) – and the same biochemical pathways to fix atmospheric nitrogen are used by both. They also have a cell wall structure similar to that of the Gram-negative bacteria, including the presence of substances known as lipopolysaccharides. These can be potent toxins in some Gram-negative bacteria (such as *Salmonella*), but in cyanobacteria they are more benign, but still present a potential public health hazard as they act as contact irritants (see Section 3.4).

Cyanobacteria commonly comprise a portion of the phytoplankton community of most freshwater bodies, including even the most pristine, although in these cases they may be only minor components. They also occur in marine (Chapter 6) and terrestrial environments.

Species from three taxonomic orders of cyanobacteria are commonly found within the freshwater phytoplankton of Australia, although species from other orders may also occur occasionally. These three orders are the Chroococcales, the Nostocales and the Oscillatoriales. The distinguishing features of each order are summarised in Table 5.1.

A commonly occurring member of the Chroococcales in Australia is *Microcystis aeruginosa* (Figure 5.1, page 130). This species is of particular concern because some strains produce a potent hepatotoxin – a toxic compound that typically attacks the liver (Falconer 2001). *Microcystis flos-aquae* (Figure 5.2, page 130) is a similar species that is also potentially toxic. There are also many tiny picoplanktonic (less than 2 μm in diameter)

Table 5.1. Summary of distinguishing features of cyanobacteria. (Classification follows that of Baker 1991, 1992).

Order	Distinguishing features	Cell shape	Typical freshwater genera
Chroococcales	Unicellular and colonial species with no physiological connection between the cells (Komárek and Anagnostidis, 1999). In colonial species, the cells are embedded within a clear mucilaginous envelope, or are located at the ends of fine, thread-like gelatinous strands that radiate from the centre of the colony. Cell numbers in colonies range from a few to many thousands.	Spherical, oval to rod shaped, depending on species, but many are coccoid	*Microcystis* *Chroococcus* *Merismopedia* *Aphanocapsa* *Aphanothece* *Coelosphaerium*
Nostocales	Multicellular filamentous species that contain some specialised cells (heterocytes, akinetes) within the filament or trichome. The filaments do not branch (false branching may occur in some genera).	The shape of the vegetative cells ranges from spherical, ovate, cylindrical to barrel shaped.	*Anabaena* *Cylindrospermopsis* *Nodularia* *Aphanizomenon* *Anabaenopsis*
Oscillatoriales	Filamentous and multicellular, but without specialised cells such as heterocytes and akinetes. The filaments are without true branching. In some genera, the filaments are enclosed within a fibrillar sheath.	The vegetative cells of some genera are often discoid – being wider than they are long – so that a filament viewed lengthwise may resemble a stack of coins. Other genera have squarish to rectangular cells. Terminal cells may differ slightly (for example, more rounded) from those within the filament.	*Planktothrix* *Planktolyngbya* *Pseudanabaena* *Spirulina* *Geitlerinema* *Planktotrichoides* *Phormidium* (mostly benthic) *Lyngbya*

species within the Chroococcales, including species from the genera *Chroococcus, Merismopedia, Aphanocapsa, Aphanothece* and *Coelosphaerium*, all of which are commonly encountered in slow flowing rivers, lakes and reservoirs.

In many parts of southern Australia, the most common problem-causing freshwater species is *Anabaena circinalis* (Figure 5.3, page 130). This cyanobacterium belongs to the Order Nostocales and may produce neurotoxins (toxins that affect the nervous system) (Baker and Humpage 1994). It was the main cyanobacterium that caused the bloom that occurred over 1000 km of the Barwon–Darling River in New South Wales in 1991 (Bowling and Baker 1996). A number of other species of *Anabaena* also occur in Australian freshwaters, including the tightly spiralled *Anabaena spiroides* (Figure 5.4, page 130). Other problem cyanobacteria from the Order Nostocales include *Cylindrospermopsis raciborskii* (Figure 5.5, page 130) – a pantropical species that produces a very potent hepatotoxin (Hawkins *et al*. 1985), and is especially common in Queensland. Another, *Nodularia spumigena*, produces yet another kind of hepatotoxin, and has been responsible for stock deaths in South Australia (Francis 1878; Codd *et al.* 1994). It is common in the freshwater sections of the lower Murray River, and also occurs in brackish through to hypersaline coastal lakes. Other genera of Nostocales commonly encountered in freshwater environments include *Cuspidothrix* (Figure 5.6, page 130), *Aphanizomenon* and *Anabaenopsis*.

No hepatotoxin- or neurotoxin-producing planktonic species of cyanobacteria from the Order Oscillatoriales have so far been reported from Australian freshwaters, although a toxic benthic species of *Phormidium* has been reported from South Australia (Baker *et al*. 2001), and toxic *Lyngbya wollei* have been recently reported from Queensland (Seifert *et al*. 2007). Other species are known to possess quite aggressive contact irritants. Toxin-producing species from the Order Oscillatoriales are, however, common elsewhere in the world, both within the phytoplankton community and growing as benthic mats on the bottom of shallow water bodies (Sivonen and Jones 1999). Common freshwater planktonic genera in Australia include *Planktothrix* (Figure 5.7, page 131), *Planktolyngbya, Pseudanabaena*, and occasionally, *Geitlerinema* and *Planktotrichoides*.

5.3 CHLOROPHYCEAE (GREEN ALGAE)

Green algae, or Chlorophyceae, are among the most numerous and diverse of all freshwater algae. At least 11 orders of green algae are recognised – and sometimes up to 19 – depending on the author. They often comprise the

BOX 5.4 DISTINCTIVE FEATURES OF CHLOROPHYCEAE (GREEN ALGAE)

Chlorophycean algae are eukaryotic organisms. The planktonic species can be present as single-celled species, as colonial species and as filamentous species. Many of the colonial species have a set number of cells per colony, with 4, 8, 16, 32 or 64 cells being present. Chlorophycean cells typically have a single nucleus and a large chloroplast in relation to the cell size. The chloroplasts can display a great variety of shapes among different genera and may also contain pyrenoids, which are associated with starch storage. Green algae contain both chlorophylls *a* and *b*, as well as carotene and xanthophyll accessory pigments. The protoplast usually fills the entire cell, but some species possess large, central aqueous vacuoles. The cell walls are generally (but not always) composed of cellulose, which is surrounded by a layer of mucilage. One group of green algae – the Order Volvocales – is normally actively motile, and swim with the aid of one, two, or occasionally four or eight flagella. All other orders have non-motile vegetative cells, but many still have a flagellated motile stage during their life cycle – either as gametes or as zoospores. Many of the non-flagellated planktonic forms have flattened colonial forms – or flattened cells with spines and other protuberances – that optimise the cell or colony's surface-area-to-volume ratio, increasing their friction against the surrounding water medium, and thus reducing their sinking rates. By this means, they remain within the circulating surface waters where they can obtain light for photosynthesis.

majority of the planktonic species of algae present in healthy freshwater ecosystems. Although some species can form blooms at times in nutrient-enriched waters, none are toxic. The Chlorophyceae are primarily a freshwater group, with about 90% of representatives occurring in freshwater environments. Attached and benthic species are common in many shallow streams and rivers, while planktonic species occur in lakes, reservoirs, ponds and other open water environments, as well as in rivers and streams (Box 5.4).

Some commonly occurring flagellated freshwater green algae belonging to the Order Volvocales include the single-celled *Chlamydomonas* and the colonial *Gonium* (Figure 5.8, page 131), *Pandorina* (Figure 5.9, page 131) and *Eudorina*, which contain small flat or spherical colonies of up to 32 or 64 cells (occasionally more), depending on species. The genus *Volvox* has hollow spherical colonies up to 2 mm in diameter that consist of several thousand small biflagellated cells. Common non-flagellated colonial green algae include *Pediastrum* (Figure 5.10, page 131) – which consists of a flat circular plate of cells that often have horn like extensions – and

Scenedesmus (Figure 5.11, page 131), which has cylindrical cells that are joined laterally in groups of four or eight. *Desmodesmus* is a similar genus where the terminal cells have spines. *Chlorella* and *Oocystis* (Figure 5.12, page 131) are also commonly found in the freshwater phytoplankton of lakes and reservoirs. These may be present as single cells, or as colonies of four to eight cells formed by the cellular division of a single parent cell, and contained within the stretched original cell wall of that parent.

The desmids are a very distinctive group of freshwater green algae, which occur either as single cells or as filaments of cells within the water column. The cells of desmids are composed of two mirror-image halves – each with a chloroplast and pyrenoids – which are joined at the centre of the cell. In many species the junction between the half cells is deeply incised to form an isthmus, and is the location of a large nucleus. Asexual reproduction is by cell division at the isthmus, with each half cell separating and growing a new half cell. Thus, one half of the desmid cell is always older than the other half. Desmids also reproduce sexually via the conjugation of two vegetative cells to form a zygote. There is a great variation in cell morphology between the common genera of desmids. *Closterium* (Figure 5.13, page 131) are frequently elongate and crescent shaped, *Cosmarium* (Figure 5.14, page 132) has an incised isthmus and hemispherical or lobed half cells, while *Micrasterias* have laterally flattened half cells with deep incisions, so that the complete cell resembles a little star. The genus *Staurastrum* contains very many different species. This genus is typified by the usual bilateral symmetry of desmids in lateral view, while in polar view the cells have tri-radial or hexa-radial symmetry. The half cells are ornamented with spines and other appendages.

5.4 BACILLARIOPHYCEAE (DIATOMS)

Diatoms are widely distributed in both freshwater and marine habitats. There are many planktonic species, but also many benthic and epiphytic (growing on plants) species as well (Box 5.5).

Many planktonic species of diatoms occur as single cells or as colonies, although some are filamentous. The most marked distinguishing feature of diatoms is their cell wall, which is composed of silica. These siliceous cell walls are composed of two overlapping halves, known as valves. One valve, the hypovalve, is smaller than the other (the epivalve), so that it fits inside the larger valve. The two valves are joined together by a girdle band that runs around the centre of the cell. When viewed under a microscope,

BOX 5.5 DISTINCTIVE FEATURES OF DIATOMS

The living cells of diatoms contain a single nucleus, and from one to many chloroplasts, the shape of which varies greatly from genus to genus. Most chloroplasts have a central pyranoid. Diatoms contain chlorophylls *a*, c_1 and c_2 as their main photosynthetic pigments, plus the accessory pigment fucoxanthin, which give the diatoms their typical golden-brown colouration. Diatom cells do not possess flagella, and thus planktonic species are reliant on turbulence within the water column to keep them from sinking. The silica cell wall is a disadvantage with regard to remaining suspended in the water column, and many planktonic species have adopted flattened or needle-like cell morphologies, spines, or colonial or filamentous growth habits, to increase their surface to volume ratio. By doing so, the cells present more resistance to the water, and sinking rates are reduced. Some non-planktonic species are, however, motile and move with a gliding motion over the substrate to which they are attached. This is done by extruding substances from their raphes.

BOX 5.6 VEGETATIVE REPRODUCTION IN DIATOMS

Vegetative reproduction involves the separation of the two valves of the parent cell, along with nuclear and protoplast division. A new valve then forms within the existing original valve that is, the new valve is always the smaller of the two. This results in the daughter cell that originated from the parental hypovalve always being slightly smaller than the parent. With continued cell division, a progressive reduction in cell size within the population occurs. Once a minimum size is reached, sexual reproduction will take place to produce an auxospore, which characteristically increases its size immediately to retain maximum size. Diatoms can also produce resting spores, which sink to the bottom and remain there until conditions for germination are suitable. Upon germination, the size increases and new vegetative cells are formed that are much larger than the original parent resting spore.

cells from the same species may look entirely different, depending on the orientation of the cell, and whether it is seen in valve view, or girdle view. There are two main forms of diatoms – centric diatoms and pennate diatoms. When viewed in valve view, centric diatoms appear circular, with radial symmetry. In comparison, pennate diatoms have long narrow cells and have bilateral symmetry when viewed in valve view. Some diatoms also have a longitudinal opening in one or both valves, known as a raphe.

In addition, the siliceous cell walls are often decorated with small holes, or pores, that may form lines or patterns on the cell wall. The cell walls may also have areas of heavy silica deposition that form strengthening ribs known as costa. The taxonomy of diatoms is based to a great degree on the pattern and structure of the cell wall. Their reproductive strategies are discussed in Box 5.6.

Some of the more common centric diatoms that occur in freshwater ecosystems include *Cyclotella* and *Coscinodiscus*, which have flattened disc shaped cells, and generally occur as single cells entrained in the water. *Aulacoseira* (Figure 5.15, page 132) is a filamentous centric diatom where the cells within the filaments appear in girdle view like miniature oil drums stacked end to end. Examples of unicellular pennate freshwater diatoms include the long skinny *Synedra* (Figure 5.16, page 132), and the spined *Urosolenia*. *Navicula* (Figure 5.17, page 132) is a genus with very many different species, both planktonic and benthic, and which typically has an elongated oval shape in valve view, and has a raphe in both valves. Colonial pennate diatoms include *Asterionella*, where one end of each of the cells are joined at a common centre to form a spoke or star-like arrangement; and *Fragilaria* (Figure 5.18, page 132), where the long narrow cells lie side by side to form rafts of cells. *Tabellaria* is another colonial freshwater pennate diatom, where the cells are joined at the corners to form zigzag chains. Some benthic species also commonly occur within the plankton community at times, especially after stormwater inflows where they have been washed off the substrate that they were growing on. These include not only small species such as the oval shaped *Cocconeis* (Figure 5.19, page 132), and also some of the large thick walled heavy species of pennate diatoms such as *Surirella* and *Pinularia* (Figure 5.20, page 132).

5.5 PYRRHOPHYCEAE (OR DINOPHYCEAE) (DINOFLAGELLATES)

The 'dinos' are also common members of freshwater phytoplankton communities, although there are fewer freshwater forms than marine species (see Chapter 6). Although some marine species are known to produce a range of different toxins, freshwater species are presently considered harmless (Box 5.7). Nevertheless, blooms can cause problems to water managers, especially of town supplies, due to the fishy tastes and odours that they produce, and by blocking water filtration equipment.

BOX 5.7 DISTINCTIVE FEATURES OF DINOFLAGELLATES

Dinoflagellates have a wide range of nutritional strategies, ranging from phototrophic, heterotrophic (consuming other cells) and saprophytic (consume dissolved organic substances). The cells of phototrophic dinoflagellates can contain several, to many, chloroplasts, which often radiate outwards from the centre of the cell. The main pigments for photosynthesis are chlorophylls *a* and c_2, but there are also several unique carotenoids present – the main one of which is peridinin. Pyrenoids are sometimes present, and starch is stored as a food reserve. The dinophycean nucleus is distinct from that of all other eukaryotic organisms in having chromosomes that are permanently condensed – and a particular form of division during cell division. Reproduction is by simple cell division. Sexual reproduction also occurs, when the zygote can form into a resting cyst. However, resting cysts can also form from vegetative cells, and are considered to be part of the natural life cycle of these organisms. Dinoflagellates also have a specialised organelle that fire projectiles if the cell is irritated. Other distinctive features of dinoflagellates are their bioluminesence and circadian rhythms.

Most freshwater dinoflagellates occur as single-celled species, although some filamentous species do exist. As the name suggests, they are typically motile – swimming with the aid of two flagella – although other variants also occur. The typical planktonic form consists of a cell that consists of an upper hemisphere (epicone) and a lower hemisphere (hypocone) that are separated by a groove that encircles the cell in its equatorial region, known as the cingulum. A second groove – the sulcus – runs transversally down the lower hemisphere from the cingulum to the pole. One flagellum encircles the cell within the cingulum; the second projects backwards from the sulcus. Many species – known as armoured dinoflagellates – have thecal plates made of cellulose that cover the entire cell. Both the number and arrangement of these plates are used to distinguish between genera and species by taxonomists. Not all dinoflagellates are armoured, however. Some – known as naked dinoflagellates – lack, or have only very thin, transparent thecal plates, but, other than this, they still display the typical cellular organisation and morphology of this division of algae.

Common freshwater genera of armoured dinoflagellates include *Peridinium* (Figure 5.21, page 132) and *Ceratium* (Figure 5.22, page 133). Naked freshwater dinoflagellates, such as *Gymnodinium* (Figure 5.23, page 133), are less common.

5.6 OTHER ALGAE

Several other groups of flagellated, motile algae – including the euglenoids (Division Euglenophyceae), cryptomonads (Division Cryptophyceae) and golden-brown algae or chrysophytes (Division Chrysophyeae) – are components of the freshwater phytoplankton. Euglenoids are common in fresh waters, especially in small ponds and farm dams where there is considerable organic pollution from animals, although members of this group also occur in brackish and marine waters. Cryptomonads also occur across a range of freshwater, brackish and marine environments, and are common components of most phytoplankton communities in lentic waters, although they are seldom present at high cell densities. In comparison, chrysophytes are a predominantly freshwater group of phytoplankton. Many species have a preference for cool, unpolluted soft waters that may be slightly acidic. They may be common in such locations, and form blooms sufficient to turn the water brown. They also tend to occur more in waters with low nutrient concentrations, rather than in phosphorus-enriched waters. Such situations include the dilute humic-acid stained coastal dune lakes of western Tasmania, and in wetlands in the coastal and tableland regions of New South Wales. They are less common in the warmer, harder waters of the Murray–Darling Basin, although they still occur as minor components of the phytoplankton communities of these ecosystems. One genus, *Dinobryon*, is however common in tropical and subtropical reservoirs. Populations may also have seasonally restricted growing seasons (Sandgren 1988b), so cells may not always be present within the phytoplankton community.

The distinctive features of euglenoids, cryptomonads and chrysophytes are provided in Boxes 5.8, 5.9 and 5.10, respectively.

Free swimming naked euglenoids typically have long cigar-shaped to oval-shaped or pear-shaped cells (such as *Euglena,* Figure 5.24, page 133), or a flattened leaf-shaped cell (such as *Phacus,* Figure 5.25, page 133) and move with a spiralling motion through the water. Their flexible cells allow them to change shape, especially under high light intensity under a microscope when they may withdraw their flagella and form into a spherical shape. When not swimming, the flexible pellicle also allows the cells to move across a surface by expanding parts of the cell while other parts contract. Armoured euglenoids – which have cells enclosed in a lorica – are typified by *Trachelomonas* (Figure 5.26, page 133).

Commonly occurring freshwater cryptomonads include *Cryptomonas* and *Rhodomonas*.

Common genera of chrysophytes that illustrate the diversity in morphology within this algal division include the unicellular *Mallomonas* and

BOX 5.8 DISTINCTIVE FEATURES OF EUGLENOIDS

Euglenoids are single-celled, motile algae. They usually have at least two flagella, but in many cases – especially in the freshwater species – only one is emergent, from a canal at the anterior end of the cell. Euglenoids often appear bright green under a microscope, due to the presence of both chlorophyll-*a* and *b*. Chlorophyll-*b* is something that euglenoids share in common with the Chlorophyceae, but not with any other division of algae. Other pigments include β carotenes and xanthophylls, which can at times give blooms of euglenoids a brick-red appearance. Many other euglenoids are colourless – lacking any photosynthetic pigmentation – and they survive by purely heterotrophic means. Even pigmented euglenoids can exhibit both photosynthetic and heterotrophic nutrition and, if placed in the dark, can lose their photosynthetic pigmentation, or become 'bleached'.

Many euglenoids are naked – lacking a cell wall as such. They do, however, contain a structure known as a pellicle just inside the exterior cellular membrane, which is composed of overlapping proteinaceous strips that wind helically around the cell, and provide considerable flexibility to change shape. There is also a group of euglenoids where the naked cells are enclosed in a non-living outer layer surrounding the cell, known as a lorica. These are often ornamented with spines, and have a short neck or pore, through which the flagella emerge.

There are often numerous disc-shaped chloroplasts scattered throughout the cells of photosynthetic species, which may have paramylon – a carbohydrate storage product – associated with them. Eyespots are present in the anterior part of the cell, near the base of the flagella. The anterior of the cell also contains a contractile vacuole that assists with osmotic regulation within the cell. The nucleus is also sometimes visible under light microscopy in the centre of the cell. Reproduction is asexual – occurring by cell division. Sexual reproduction has yet to be demonstrated. Some euglenoids can form cysts to withstand periods of unfavourable environmental conditions. Some species also have phototaxic circadian rhythms, moving up and down the water column in response to light and at times, forming scums on the surface of the water. Common genera include *Euglena Phacus*, *Lepocinclis*, *Trachelomonas* and *Strombomonas*.

Synura, which forms spherical to ovate colonies. Both genera have small siliceous scales and some species have spines or bristles. Another genus, *Dinobryon*, has cells enclosed in loricas and which form linear or branching colonies.

BOX 5.9 DISTINCTIVE FEATURES OF CRYPTOMONADS

The cells of cryptomonads are flattened, giving them a bean- or heart-shaped appearance when viewed from the side. They are mainly single-celled, free-living and highly motile flagellates – having two flagella, one of which may be slightly shorter than the other. These typically emerge from a ventrally located depression or gullet, which, if present, opens towards the anterior end of the cell. The gullet is often lined with small organelles known as ejectosomes, which are discharged when the cell experiences some disturbance, unreeling long threads. These ejectosomes also occur on other parts of the cell.

The cells of cryptomonads are naked – lacking a cell wall. The cell itself most usually contains either one or two chloroplasts. In most cells, a single chloroplast is present, which contains two lobes joined in the middle by a pyrenoid. Cryptomonads possess both chlorophyll-*a* and c_2, plus several other distinctive accessory pigments including carotenes, xanthophylls, phycocyanin and phycoerythrin. Cryptomonads can therefore display a variation in colouration, including red, blue, yellow, brown and green. Some are colourless (as they lack a chloroplast), and are heterotrophic. Starch is the main storage product. Asexual reproduction occurs with the cell dividing longitudinally, but no sexual reproduction has been recorded.

BOX 5.10 DISTINCTIVE FEATURES OF CHRYSOPHYTES

Planktonic chrysophytes are motile, and swim with the aid of two flagella – although in many species the second of these may be reduced to only a short stub. An eyespot may be present in the cell near the base of the flagella. Some chrysophytes may also undergo diurnal migrations up and down the water column of water bodies, indicating they may be responsive to light availability within the water body. In general, planktonic chrysophyte cells are ovate to tear drop in shape. The outside of the cell varies considerably, with some genera being naked – with nothing covering the cell membrane – while other genera have coverings of ornate siliceous scales and spines and, in yet others, the cells are contained within a funnel- or urn-shaped lorica secreted by the cell itself. There may be one or a few chloroplasts present within the cell. Chrysophyte pigmentation includes chlorophyll-*a* and both c_1 and c_2, and also fucoxanthin, which gives the typical golden-brown colour. Pyrenoids occur within the chloroplasts, and the cells contain a storage product know as chrysolaminarin. In addition to being photosynthetic, many chrysophytes have been shown to also be heterotrophic – actively ingesting bacteria, and even other algae. The chrysophyte nucleus is located in the anterior section of the cell. Asexual reproduction takes place through the binary fission of cells. Sexual reproduction has been reported for only a few species, with two vegetative cells fusing to form a zygote. Chrysophyte vegetative cells can also form resting cysts, which have ornamented siliceous external walls.

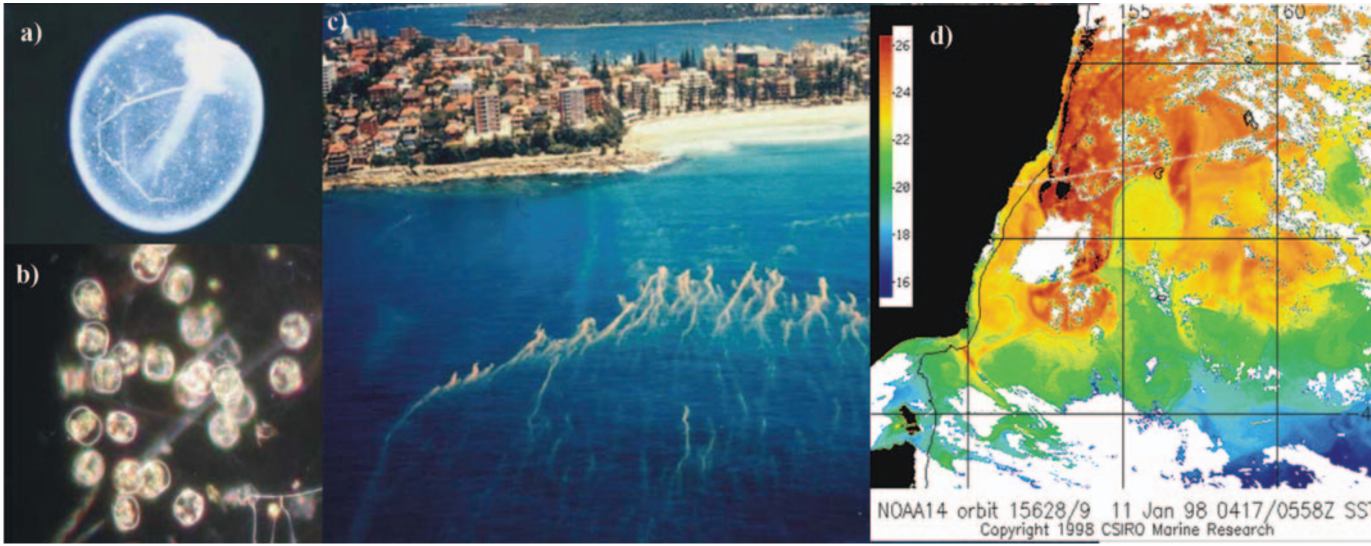


Figure 3.2 (a) Dark-field light microscopy images of a red-tide-forming dinoflagellate *Noctiluca scintillans* (100–1000 μm diameter), and (b) the prey – the chain-forming diatom *Thalassiosira* (2–86 μm diameter); (c) aerial photo of a *Noctiluca* bloom off Manly in Sydney, 1990s Beachwatch; (d) sea surface temperature image depicting EAC in summer, 2003 *NOAA/CSIRO Marine Research*. (a–c courtesy of NSW Department of Environment and Climate Change.)

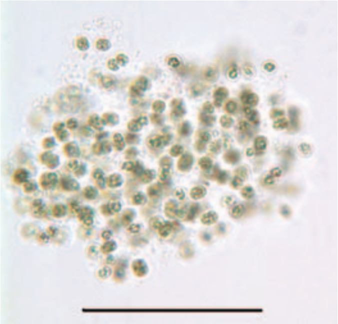


Figure 5.1 Colony of *Microcystis aeruginosa*. Note almost spherical cells – often in doublets – within a gelatinous matrix. Scale bar 50 µm.

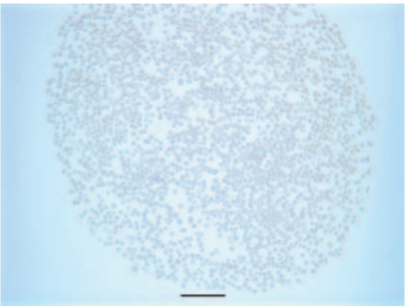


Figure 5.2 Colony of *Microcystis flos-aquae*. Similar to *M. aeruginosa*, but cells are generally more dispersed within the gelatinous matrix, which has a more compact shape. Scale bar 50 µm.



Figure 5.3 Filament of *Anabaena circinalis*. Note the specialised cells – known as heterocytes – within the filament. These are sites of nitrogen fixation. Scale bar 50 µm.



Figure 5.4 Filament of *Anabaena spiroides*, also with heterocytes. Compare the tight spirals with the open spirals of *A. circinalis*. Scale bar 50 µm.

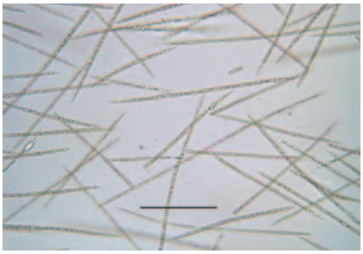


Figure 5.5 Filaments of *Cylindrospermopsis raciborskii*. The specialised cells within the filaments are akinetes (resting spores). Tiny conical heterocytes occur at the ends of some filaments. Scale bar 50 µm.

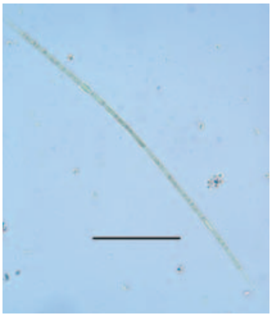


Figure 5.6 A filament of *Cuspidothrix issatascheenkoi* containing heterocytes. The terminal cells are long, tapering and colourless. Scale bar 50 µm.

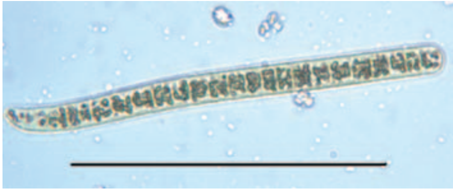


Figure 5.7 A filament of *Planktothrix isothrix*, with rounded terminal cells. Scale bar 50 μm.

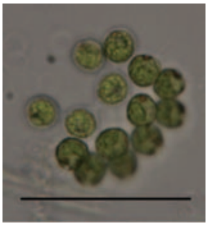


Figure 5.8 Part of a colony of *Gonium* sp. showing the almost spherical biflagellated cells in a flat plate arrangement. Scale bar 50 μm.

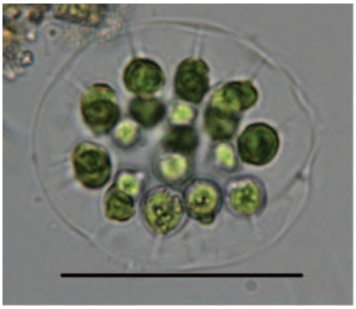


Figure 5.9 Colony of *Pandorina* sp. The colony has a spherical structure with the flagella of each cell radiating outwards. Scale bar 50 μm.

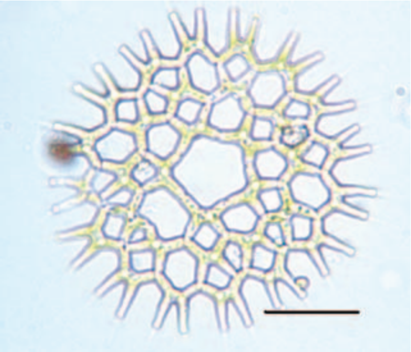


Figure 5.10 A colony of *Pediastrum duplex*, composed of approximately X- or H-shaped cells joined at the tips. Scale bar 50 μm.

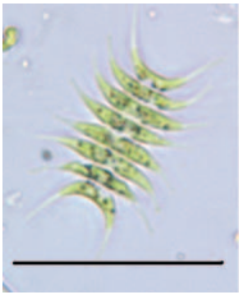


Figure 5.11 *Scenedesmus dimorphis* – a colonial green alga composed of eight crescent-shaped cells. Scale bar 50 μm.

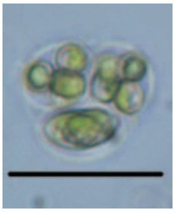


Figure 5.12 Colonies of ovoid-shaped *Oocystis* sp. cells. Three new colonies are contained within the original parent cell wall. Scale bar 50 μm.

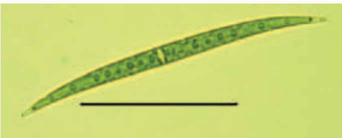


Figure 5.13 *Closterium* sp. – a crescent-shaped desmid. Note the two half cells with large chloroplasts containing pyrenoids. Scale bar 50 μm.

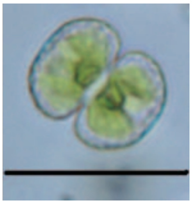


Figure 5.14 *Cosmarium* sp. – a desmid with two distinct half cells joined at a central isthmus. Scale bar 50 µm.

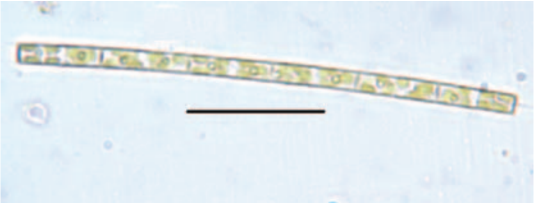


Figure 5.15 A filament of the diatom *Aulacoseira* sp. Note the number of chloroplasts within each cell. Scale bar 50 µm.

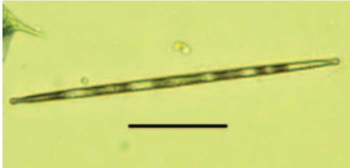


Figure 5.16 *Synedra* sp. – a long, needle-shaped, pennate diatom. Scale bar 50 µm.

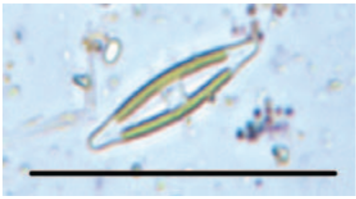


Figure 5.17 A small cell of *Navicula* sp. There are several hundred of species within this genus. Scale bar 50 µm.

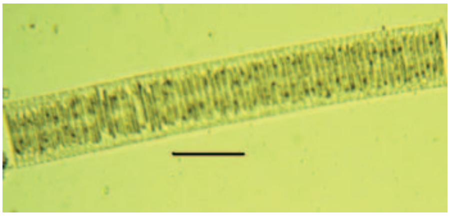


Figure 5.18 A colony of the diatom *Fragilaria* sp. The pennate shaped cells join together lengthwise to form a raft of cells. Scale bar 50 µm.

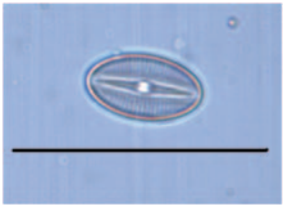


Figure 5.19 A small ovoid shaped cell of *Cocconeis* sp. – in valve view – illustrating the patterned silica cell wall. Scale bar 50 µm.

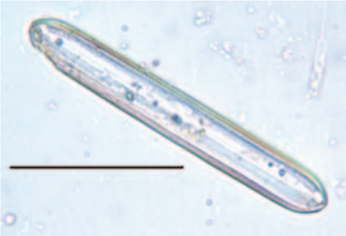


Figure 5.20 A cell wall from *Pinularia* sp. These are large diatoms that have a heavy silica cell wall and are usually found in benthic habitats. Scale bar 50 µm.

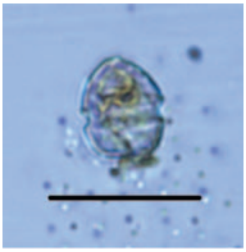


Figure 5.21 A small cell of *Peridinium* sp., illustrating the epicone, hypocone and cingulum. Scale bar 50 µm.



Figure 5.22 *Ceratium hirundinella* – a large dinoflagellate often found in nutrient enriched waters, which can cause fishy tastes and odours and block filtration equipment in town water supplies. Scale bar 50 µm.



Figure 5.23 *Gymnodinium* sp. – a naked dinoflagellate. Note the cingulum and the multiple chloroplasts within the cell. Scale bar 50 µm.



Figure 5.24 *Euglena* sp., showing numerous small disc-shaped chloroplasts and other internal structures. Scale bar 50 µm.

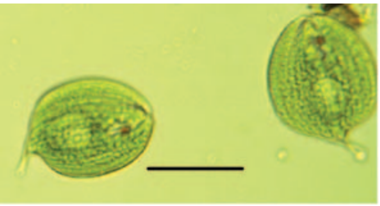


Figure 5.25 *Phacus* sp. – a flattened leaf shaped euglenoid. Scale bar 50 µm.



Figure 5.26 A cell of *Trachelomonas* sp. – an armoured euglenoid. Scale bar 50 µm.

(Figures 5.1–5.26 are courtesy of Water Environment Laboratory, NSW Department of Water and Energy.)

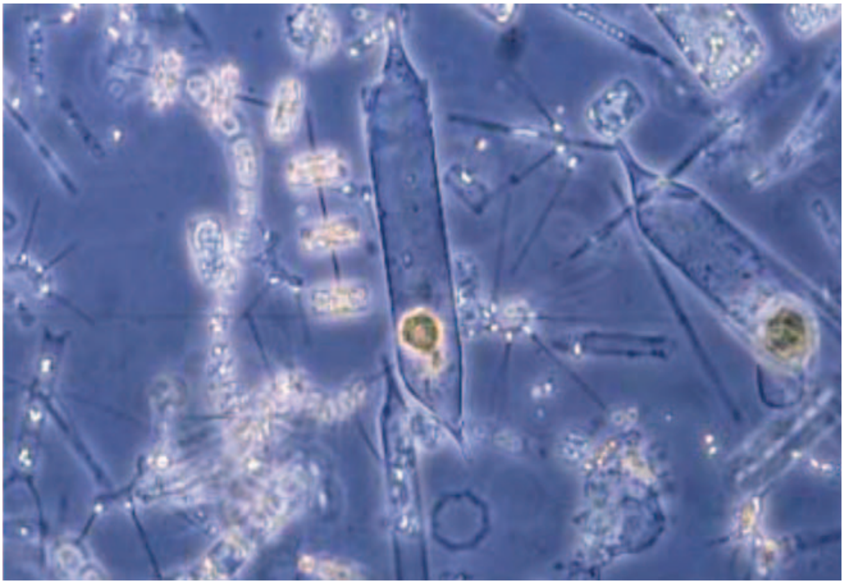


Figure 6.1 Common diatom species found in temperate coastal waters of New South Wales (*Chaetoceros* spp., *Thalassiosira* spp., *Rhizosolenia* spp. and *Astrionellopis* spp.). Width of photo is approximately 60 μm.

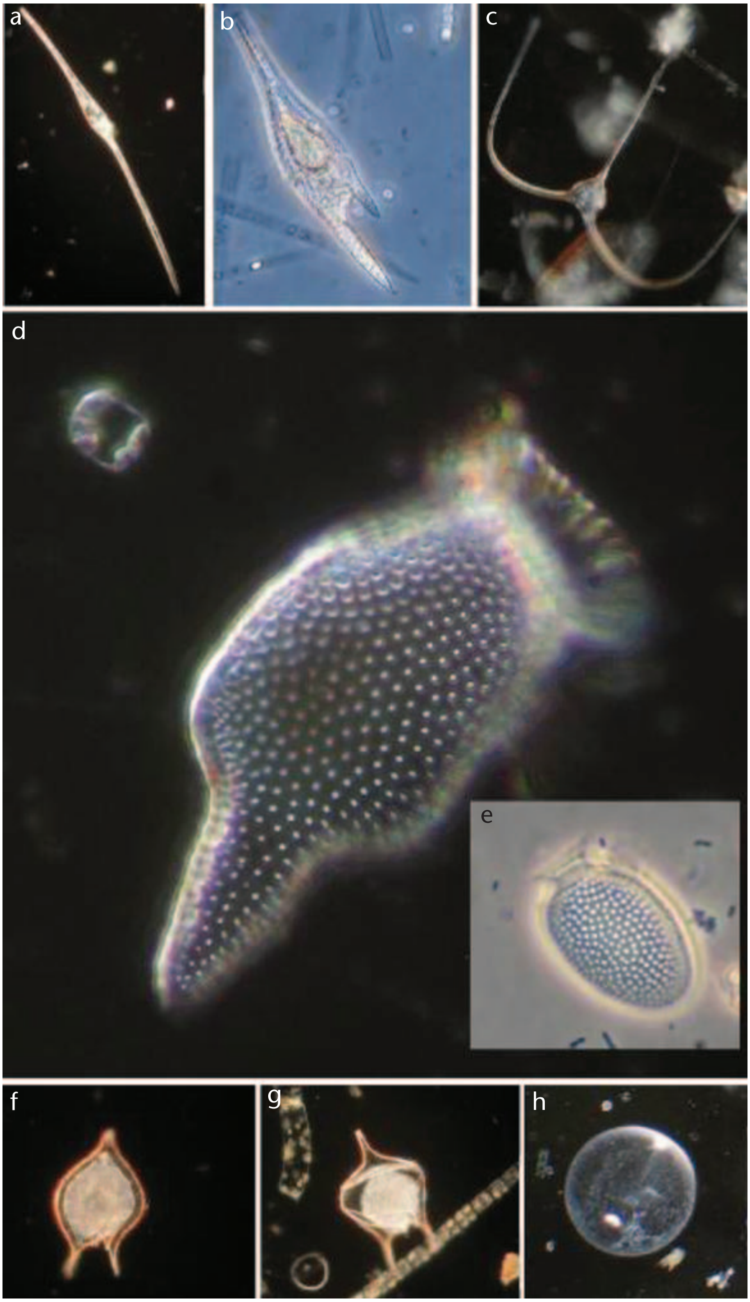


Figure 6.2 Common dinoflagellate species found in temperate coastal waters of New South Wales (a–c) *Ceratium* spp., (d, e) *Dinophysis* spp., (f, g) *Protoperidinium* spp. and (h) *Noctiluca scintillans*.

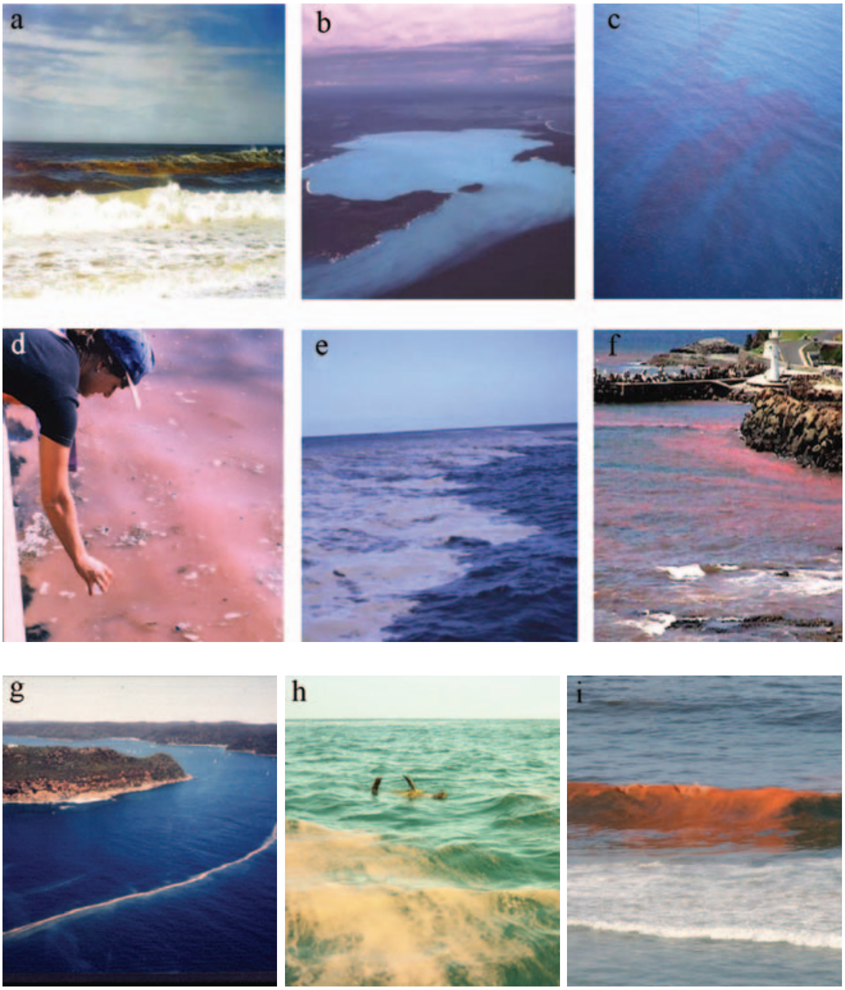


Figure 6.5 Common water discolorations caused by algal blooms in New South Wales marine and estuarine waters (a) *Anaulus australis*, (b) *Gephrocapsa oceania* (Blackburn and Cresswell 1993), (c) *Mesodinium rubrum*, (d) *Noctiluca scintillans*, (e) *Trichodesmium erythraeum* and (f–i) *Noctiluca scintillans*.

5.7 CONCLUSIONS

There is considerable diversity found among freshwater phytoplankton. At least seven algal divisions are commonly represented within freshwater phytoplankton communities – each differing from the other in their cellular structure, pigment arrays and the presence or absence of motile structures such as flagella. Within each division there is further variability. Examples of this include:

- the three commonly found orders of cyanobacteria
- the great diversity within the green algae, including both flagellated and non-flagellated forms
- the centric and the pennate forms of diatoms
- the armoured and naked forms of dinoflagellates and euglenoids.

Superimposed on this is the variation in growth form throughout the cell cycle, with single-celled, filamentous and colonial species within many of the divisions.

Freshwater phytoplankton are an integral part of all freshwater ecosystems, with representatives found from pristine to polluted water bodies. They contribute to the food webs of these systems, along with benthic algae, other aquatic macrophytes and inputs from terrestrial sources. In most systems, freshwater phytoplankton do not cause environmental problems. It is only when conditions are suitable for explosive growth, such as an excess in nutrients, that algal blooms cause water-quality problems that may affect both the ecosystem in which this occurs and anthropogenic uses of the water. Of all the types of freshwater phytoplankton that may bloom, the cyanobacteria are of most concern because of the potential hazard these create through the ability of some species to produce potent toxins. Because of this, considerable effort must be put into sampling freshwater phytoplankton communities – especially for public health surveillance – and adequate sampling methods must be employed to obtain a representative measure of phytoplankton presence within particular water bodies.

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Chapter 6

Coastal and marine phytoplankton: diversity and ecology

Penelope Ajani and David Rissik

6.1 IDENTIFYING MARINE PHYTOPLANKTON

Phytoplankton consist of microscopic algae (*phyto* = *plant*) that live suspended in the water (*planktos* = made to wander). With more than 10 000 species identified in coastal and oceanic waters, algae are a varied group with up to thirteen divisions. They range in size from 0.2 to 200 µm, with a few taxa attaining up to 4 mm in length. Most phytoplankton species are able to produce their own energy (they are primary producers) by converting solar energy and nutrients into chemical energy in the form of carbohydrate, using photosynthesis. A by-product of this process is the production of oxygen and it is considered that at least half of the oxygen in the atmosphere is produced by phytoplankton. The vast abundance of phytoplankton provides nutrition – either directly or indirectly – for all other forms of marine life. Certain algae, however, are not true plants because they lack photosynthetic pigments and must eat other cells, but are classified as algae because of their close resemblance to photosynthetic forms.

Pigments are chemical compounds that absorb certain wavelengths of visible light and reflect the other colours that we see. They absorb a narrow

Table 6.1. Important pigments found in major algal groups.

Pigments	Cyanobacteria (Blue-green algae)	Dinophyta (Dinoflagellates)	Bacillariophyta (Diatoms)	Chrysophyta, Raphidophyceae	Chrysophyta, Dictyophyceae	Prymnesiophyta (Haptophytes)	Chryptophyta (Chloromonads)	Euglenophyta (Euglenoids)	Chlorophyta (Prasinophytes, Chlorophytes)
Chlorophylls									
Chlorophyll-*a*	X	X	X	X	X	X	X	X	X
Chlorophyll-*b*		X*						X	X
Chlorophyll-*c1*			X	X	X	X			
Chlorophyll-*c2*		X	X	X	X	X	X		
Chlorophyll-*c3*			X		X	X			X
Phycobilins									
Phycocyanin	X						X		
Allophycocyanin	X								
Phycoerythrin	X						X		
Phycobilisomes	X	X*							
Carotenes									
α-carotene							X		
β,β-carotene	X	X	X	X	X	X		X	X

Pigments	Cyanobacteria (Blue-green algae)	Dinophyta (Dinoflagellates)	Bacillariophyta (Diatoms)	Chrysophyta, Raphidophyceae	Chrysophyta, Dictyophyceae	Prymnesiophyta (Haptophytes)	Chryptophyta (Chloromonads)	Euglenophyta (Euglenoids)	Chlorophyta (Prasinophytes, Chlorophytes)
Xanthophylls									
Zeaxanthin	X								
Echinenone	X								
Canthaxanthin	X								
Myxoxanthophyll	X								
Oscillaxanthin	X								
Peridinin		X							
Dinoxanthin		X							
Diadinoxanthin		X	X	X		X			
Fucoxanthin		X*	X	X	X	X			
Diatoxanthin			X			X			
Violaxanthin				X					X
Lutein									X
Alloxanthin							X		
Neoxanthin								X	X

*Through symbiosis with other groups.

range of wavelengths of light capturing the energy of sunlight for photosynthesis. In order to acquire more of the sun's energy, photosynthetic organisms such as phytoplankton produce several kinds of pigments to absorb a broader range of wavelengths. This difference in pigment combinations is reflected in the names of the taxonomic divisions of algae, as well in as their evolutionary relationships (Table 6.1). The response of pigments to particular light wavelengths also provides us with a method of measuring plankton biomass, and distinguishing between the biomass of major phytoplankton groups. It can even help to determine the production rates (growth) of phytoplankton communities.

In the following sections we will discuss the major groups of phytoplankton found in temperate coastal waters and give a brief description of each group:

- Bacillariophyceae (diatoms, Section 6.2)
- Dinophyceae (dinoflagellates, Section 6.3)
- Cyanophyceae (blue-green algae, Section 6.4)
- Others (Section 6.5)
  - Chrysophyceae, Class Raphidophyceae (chloromonads)
  - Chrysophyceae, Class Dictyochophyceae (silicoflagellates)
  - Prymnesiophyceae = Haptophyceae (coccolithophorids, prymnesiophytes, golden brown flagellates)
  - Chryptophyceae (chryptomonads)
  - Euglenophyceae (green flagellates)
  - Chlorophyceae (prasinophytes, chlorophytes)

Phytoplankton are classified into taxonomic groups based on the combinations of their photosynthetic pigments, as well as other characteristics such as the way in which they store energy (lipid or carbohydrate) and the structure of their cell walls. Other distinguishing features include:

- the presence or absence of flagella
- the structure of the flagella or flagella roots
- the pattern and course of mitosis (cellular division) and cytokensis (cell division)
- other morphological attributes such as symmetry and size.

Many of these groups are represented in the microplankton (20–200 μm), the nanoplankton (2–20 μm) and the picoplankton (0.2–2 μm) – with some occurring in all three size classes. In temperate coastal waters, the nanoplankton can account for 80% of the total phytoplankton biomass,

while in tropical waters the picoplankton can account for 80% of the total phytoplankton biomass. Green flagellates, small non-thecate dinoflagellates, cryptomonads, prymnesiophytes, coccolithophorids and other colourless flagellates are all common representatives of the nanoplankton in our waters. Picoplankton are represented by the cyanobacteria and chrysophytes.

6.2 DIATOMS (DIVISION BACILLARIOPHYCEAE)

Diatoms are unicellular (but often live in colonies and some form chains), microalgae with membrane-bound cell organelles and which have a siliceous cell wall or frustule, which is made up of two parts (known as valves) – the hypovalve and epivalve. The structure and patterns and processes of the cell wall form the basis for the two major groups within the diatoms (pennate and centric diatoms). Pennate diatoms are elongate and usually bilaterally symmetrical, with up to 800 marine species identified. Centric diatoms are usually round or 'radially symmetrical' (with the frustule often compared to a Petri dish or pillbox) and there are up to about 1000 species in marine waters (Figure 6.1, page 134).

Diatoms (unlike nearly all other phytoplankton) have no flagella and are in most cases non-motile. Pennate forms can achieve a gliding motion via mucilage secretion through their raphe system (a longitudinal slit in the valve) while centric diatoms can exude mucilage through their labiate process (a tube or opening through the valve wall), allowing limited movement.

Diatoms can also be found as benthic forms growing on sediments, rocks and plants (Box 6.1). In coastal waters, limiting factors – such as silicate (and other nutrient) availability, water stability, light climate, parasitism and grazing – affect which species are present in the water column at particular times (Table 6.2). Diatom blooms often occur along in coastal waters when episodic upwelling brings nutrient-rich water to the surface, where there is better access to light and subsequent increased production (Box 6.2).

Some diatom blooms can become so dense that they can cause death to fish and invertebrates due to either oxygen depletion or by abrasion damage to their gills (such as *Thalassiosira* spp. and *Chaetoceros* spp.). Species belonging to the genus *Pseudo-nitzschia* have been implicated as the causative organisms of amnesic shellfish poisoning (Box 6.3).

Diatom frustules have a slow rate of decay, which has resulted in massive geological deposits known as diatomaceous earth (which is used in filtration, cosmetics, toothpaste and even forensic science).

BOX 6.1 BENTHIC MICROALGAE

Benthic microalgae or microphytobenthos (mpb) are important communities in terms of estuarine and coastal ecology. Mpb assemblages play a central role in the production and cycling of organic matter in these environments as well as stabilising sediments by excreting mucilaginous substances into the sediment and thus preventing erosion.

These assemblages usually include bacteria, flagellates, ciliates, diatoms, dinoflagellates and other algae, as well as foraminifers and nematodes. Further groupings can be found within the diatoms – some live freely on (epipelic) or in the sediments (endopelic). Those living attached to the substratum are classified according to their substrata preference – sand grains (epipsammic), rock or stones (epilithic), plants (epiphytic) and epizoic (animals).

Although phytoplankton communities in coastal waters have received much attention, very few studies have been carried out on the mpb communities. This is probably because of the difficulties in extracting and enumerating the microbes from the sediments. The few studies that have been carried out in our coastal waters list the abundant mpb genera as the diatoms *Amphora, Navicula, Nitzchia, Gyrosigma* and *Pleurosigma* as well as the dinoflagellates *Amphidinium* and *Prorocentrum*. Green euglenoids, such as *Eutreptia*, are also common.

6.3 DINOPHYCEAE (DINOFLAGELLATES)

Dinoflagellates are a group of unicellular algae with membrane bound organelles (they are eukaryotes) and flagella. There are approximately 2000 living species known (130 genera, Figure 6.2, page 135). About half the dinoflagellates feed on organic matter only (that is, they are heterotrophs, including some carnivores) and the other half either photosynthesise or are partly autotrophic and partly heterotrophic (that is, part animal, part plant).

Dinoflagellates are motile at some stage of the lifecycle – having two different flagella. One flagellum is situated in a girdle groove around the middle of the cell (for rotation) and the other projects from the sulcus groove (at one end) for propulsion. Careful use of a microscope is required to see these flagella.

Dinoflagellates may be armoured (thecate – with cellulose cell walls made of plates) or unarmoured (non-thecate). Armoured dinoflagellates are usually irregular in shape, bearing horns, ridges and wings.

Over 80 species of marine dinoflagellates are known to produce cysts (more than 16 of these species are known to cause red tides and seven

Table 6.2. Factors affecting the growth, abundance and species composition of phytoplankton populations (adapted from Jeffrey and Hallegraeff 1990).

Physical	• Temperature – growth is possible within range; effect on rate of growth, on nutrient demands and on enzymatic processes; thermal stratification • Light – length and brightness of day; spectral composition; light saturation; inhibitory or lethal intensities; IR absorption; UV effects • Water movements – horizontal and vertical transport into and out of an area or depth zone; invasions; eddy diffusion • Density distribution – effects of salinity, temperature, metabolism or gas production in relation to the sinking or rising rate (buoyancy) of organisms
Chemical	• Inorganic substances – nitrogen compounds, phosphates, silicates, sulphides, iron, trace elements, oxygen, ironic ratios and salinity, redox potentials, pH • Organic substances – vitamins (B_{12}, biotin and thiamine), acids (glycolic and glutamic), chelates, unknown or imperfectly known compounds such as 'humus', natural chelates and most extracellular compounds • Light-absorptive capacity of algal pigments
Biological	• Inhibitory or stimulatory substances – through the activities of previous populations or the organisms own extracellular products (e.g. lag phases, toxin production) • Intrinsic factors (phased cell division, diurnal and circadian rhythms); regenerative strategies (i.e. seeding ability) • Cellular organisation and nutrition • Life histories, reproductive strategies, resting stages • Resource competition in relation to growth • Symbiosis – bacteria on algal cells or in their mucilage; algal cells within algal cells • Grazing pressure from zooplankton – quantitative and qualitative effects • Parasitism • Morphological diversity – cell structure (unicellular, colonial, filamentous); surface to volume ratios; mobility

species to be toxic). Cysts can be of two types – either temporary cysts (that is, the cell quickly re-established itself after a brief encystment) or resting cysts, which sink from the water column and often remain in the sediment anywhere from 6 weeks to 5 months, depending on the species.

BOX 6.2 THE 'SURF DIATOM': *ANAULUS AUSTRALIS*

The 'surf diatom' – *Anaulus australis* – has been reported as oily slicks at various NSW beaches. These cells are able to rise to the surface and form dense accumulations by attaching themselves to wave-generated bubbles in high-energy surf zones. In most cases, these accumulations disappear at night and reappear each morning. This species has been reported along the southern coasts of South Africa and Australia (McLachlan and Hesp 1984; Campbell 1996).

BOX 6.3 SPECIES IN THE *PSEUDO-NITZSCHIA* GENUS

Species belonging to the genus *Pseudo-nitzschia* have been implicated as the causative organisms of amnesic shellfish poisoning (UNESCO 1995; Hallegraeff 1994). Blooms of the toxic species *P. multiseries* (5% of total phytoplankton biomass) were detected over a 2-year period in Berowra Creek – in northern Sydney. A bloom predominantly of *P. pseudodelicatissima* (Figure 6.3h) has also been detected in Berowra Creek. Although this species has been found to be toxic elsewhere (UNESCO 1995), analysis results from oysters from nearby leases showed no detectable concentrations of domoic acid. Oyster leases in Wagonga Inlet, Narooma, have been closed for harvesting due to a bloom of *P. pseudodelicatissima, P. pungens* and *P. australis* (toxic species).

The purpose of cyst formation is probably a survival strategy, which is regulated by both physiological and environmental factors such as:

- protection from adverse conditions (such as temperature or nutrient availability)
- a refuge from predation
- alternation between planktonic and benthic habitats
- as part of the reproductive process
- to aid in dispersion/seed population for the subsequent bloom.

Many dinoflagellates make daily diurnal migrations up and down the water column. During the day they migrate towards the surface of the water (for better light availability) and at night they move down to a depth of several metres (for better access to nutrients). This vertical migration is an important consideration when sampling or when analysing the results of sampling activities.

A regularly occurring red-tide on the south-east Australian coast is caused by the dinoflagellate *Noctiluca scintillans* (Figure 6.3b). *Noctiluca* are large (0.2–0.8 mm diameter) balloon-shaped, heterotrophic dinoflagellates, which consume other algae, some zooplankton and even fish eggs. They have no photosynthetic pigments, although in tropical waters they may appear green due to endosymbiotic flagellates. As *Noctiluca* blooms die off, the cells float to the surface forming dense red slicks. Ammonia stored as a waste product is often released at this stage, which is potentially dangerous to fish. *Noctiluca* are bioluminescent (they glow) at night, especially around a moving boat or breaking wave. Interestingly, the frequency of observation of this species off south-eastern Australia has increased during 1970s to 1990s. This may be due to a number of reasons, including a response to coastal eutrophication (Ajani *et al.* 2001a).

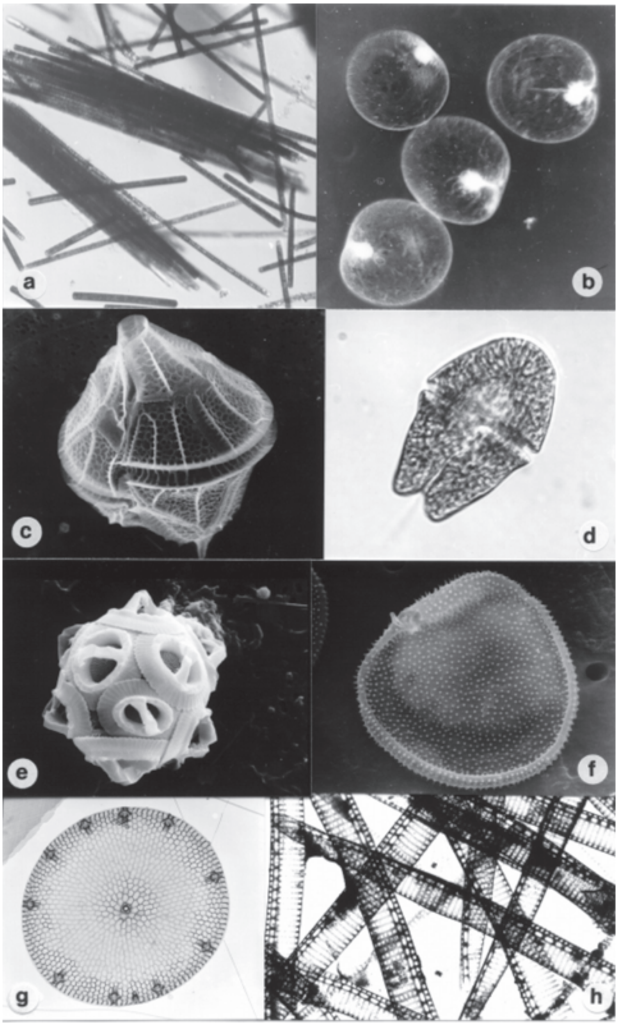


Figure 6.3 Common bloom species in New South Wales marine and estuarine waters. a) LM of the filamentous cyanobacterium *Trichodesmium erythraeum* producing raft-like bundles, up to 750 µm long, b) LM of the balloon-shaped, colourless dinoflagellate *Noctiluca scintillans,* 200–500 µm diameter, c) SEM of the dinoflagellate *Gonyaulax polygramma,* showing ornamented cellulose plates with longitudinal ridges, 29–66 µm long, d) LM of the large, unarmoured dinoflagellate *Akashiwo sanguinea,* 50–80 mm long, e) SEM of the calcareous nanoplankton *Gephyrocapsa oceanica,* 15 µm diameter, f) SEM of the triangular, armoured dinoflagellate *Prorocentrum cordatum,* 10–15 µm wide and covered with minute spinules, g) TEM of the weakly silicified cell of the centric diatom *Thalassiosira partheneia,* 10 µm diameter, h) TEM of the pennate diatom *Pseudo-nitzschia pseudodelicatissima,* 57–150 µm long. (NSW DECC.)

> **BOX 6.4 *DINOPHYSIS ACUMINATA***
> *Dinophysis acuminata* (Figure 6.4g) – a producer of diarrhetic shellfish poisoning (DSP) – was implicated in the contamination of edible bivalves at two locations on the east coast of Australia, with over 80 cases of gastroenteritis being reported. A mouse bioassay revealed a positive result for an unidentified DSP toxin and *D. acuminata* were found in the guts of the bivalves. Pectenotoxin DSP toxins have now been fully characterised. Peak concentrations of *D. acuminata* at the Port Hacking 100 m station off Sydney generally occur in January (Ajani *et al.* 2001a).

Dinoflagellates have the largest number of harmful species (around 40 species). They can produce toxic compounds that accumulate in filter-feeding bivalves and commercially important crustaceans and finfish. Consumption of these fisheries by humans can result a range of symptoms including gastroenteritis, headaches, muscle and joint pain, and, in extreme cases, paralysis and respiratory failure (PSP, DSP, NSP and ciguatera poisoning, Box 6.4). On a global scale, over 2000 cases of human-poisoning through fish or shellfish consumption are reported each year (Hallegraeff 1995).

6.4 CYANOBACTERIA (BLUE-GREEN ALGAE)

Cyanobacteria are primitive algae characterised by the absence of the membrane-bound cell components (they are prokaryotes). Cyanobacteria are often blue-green in colour. They have unicellular, colonial and filamentous forms and do not have flagellate cells at any stage in their life cycle.

Blue-green algae include benthic and planktonic forms. Many species have adaptations to aid survival in extreme and diverse habitats, such as gas vacuoles for buoyancy control, akinetes (resting stages) and heterocysts (specialised cells which can fix atmospheric nitrogen) for survival in waters where the nitrate and ammonia levels are relatively low. Not all taxa have these features. In marine and brackish waters, blue-green algae have produced toxins that have resulted in neuromuscular and organs distress as well as external contact irritation.

Six genera of blue-green algae have been implicated in blooms in Australian coastal waters: *Anabaena, Microcystis, Amphizomenon, Nodularia, Trichodesmium* and *Lyngbya. Trichodesmium erythraeum* is the most common blue-green in temperate coastal waters of NSW (Box 6.5). This tropical/subtropical species produces episodic 'red tides' that were historically reported as 'sea sawdust' during Captain Cook's voyage through the

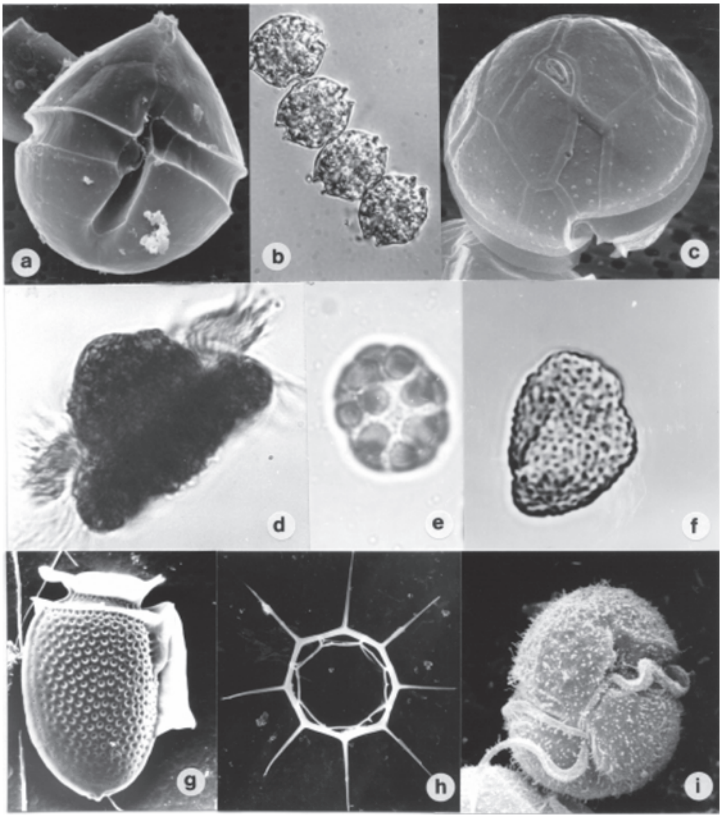


Figure 6.4 Common bloom species in New South Wales marine and estuarine waters. a) SEM of the red-water dinoflagellate *Scripsiella trochoidea,* 16–36 µm long. Note tube-shaped apical pore on top of the cell and nearly equatorial (not displaced) girdle groove, b) LM of the chain-forming dinoflagellate *Alexandrium catenella* – the causative organism of paralytic shellfish poisoning. Individual cells 20–22 µm long, c) SEM of the red water dinoflagellate *Alexandrium minutum* – the causative organism of paralytic shellfish poisoning. Individual cells 24–29 µm diameter. Note the hook-shaped apical pore on top of the cell and characteristic shape of the first apical plate, d) LM of the ciliate *Mesodinium rubrum,* with two systems of cilia arising from the waist region, 30 µm diameter, e) LM of the 'raspberry-like' cell of the fish-killing flagellate *Hetersosigma akashiwo* ('Akashiwo' = red tide), containing numerous disc-shaped chloroplasts, cell 11–25 µm long, f) LM of an undescribed flagellate resembling *Haramonas*. The cell surface is covered by numerous mucous-producing vesicles, cells 30–40 µm long, g) SEM of the small armoured dinoflagellate *Dinophysis acuminata* – the causative organism of diarrhetic shellfish poisoning, cells 38–58 µm long, h) SEM of the siliceous skeleton of the silicoflagellate *Dictyocha octonaria,* 10–12 µm diameter, i) SEM of the small unarmoured, fish-killing dinoflagellate *Karlodinium micrum,* 15 µm diameter. (NSW DECC.)

> **BOX 6.5 *TRICHODESMIUM ERYTHRAEUM***
> A particularly large bloom of *T. erythraeum* occurred once off southern New South Wales. Sea-surface-temperature imagery showed the bloom was associated with unusually warm water throughout the area. Perhaps strong warming from the East Australian Current transported and triggered the bloom in local estuaries such as Batemans Bay, Clyde River estuary. The association with warm water is evident in this species' annual distribution (Ajani *et al.* 2001a) from the Port Hacking 100 m station (off Sydney), where peak concentrations occur in mid-April when surface waters exceed around 22°C.

Coral Sea in 1778. The filaments of this alga are united (parallel) into small raft-like bundles that are just visible to the naked eye (around 1 mm). The filaments are generally cylindrical, uniformly broad or slightly tapering at the tips, and are straight or slightly curved. *Trichodesmium* filaments do not have any specialised cells such as heterocysts or akinetes (Figure 6.3a).

Blooms of *Trichodesmium erythraeum* are most commonly seen in northern NSW waters in spring, summer and early autumn when the East Australian Current (EAC) transports these algal masses into NSW from Queensland waters (Box 6.5). These blooms appear yellow-grey in their early stages, while they become a reddish-brown later (Figure 6.5e, page 136).

6.5 OTHER MARINE PHYTOPLANKTON

6.5.1 Chrysophyceae

Class Raphidophyceae (Chloromonads)

Chloromonads/raphidophytes are unicellular flagellates that have two unequal, heterodynamic flagella arising from a sub-apical shallow groove. The forward-directed flagellum has two rows of fine hairs, while the trailing flagellum is smooth and lies close to the surface of the cells. Their cells are unarmoured), dorsoventrally flattened (potato-shaped) and contain numerous ejectosomes, trichocysts and/or mucocysts that readily discharge upon stimulation (they have a characteristic 'raspberry-like' appearance upon disintegration, which can make identification difficult) (Figure 6.4e). Many raphidophytes can be toxic to fish and bloom events have been reported throughout the world in coastal and estuarine waters (Box 6.6). *Heterosigma, Chatonella* and *Fibrocapsa* commonly bloom in summer.

BOX 6.6 TOXIC RAPHIDOPHYTE BLOOMS

A toxic raphidophyte, *Chatonella* cf. *globosa*, bloomed sporadically in Canada Bay, Sydney Harbour on a few occasions. Blooms of related species have caused significant mortality of cultured yellowtail and red sea bream in Japanese inland seas (Okaichi 1985) and implicated in the mass mortality of farmed blue-fin tuna in Boston Bay, South Australia (Marshall and Hallegraeff 1999). The production of superoxide radicals as the major mechanism of fish mortality is also hypothesised for this genus. Evidence for brevetoxin-like production is still being investigated.

BOX 6.7 SILICOFLAGELLATE BLOOMS

A silicoflagellate, *Dictyocha octonaria* (Figure 6.4h), was implicated as the causative organism in a fish kill which occurred in coastal waters off Newcastle. Dead fish (especially *Caranx* sp.) were seen on beaches between Burwood Beach and Redhead and floating up to 3 km offshore. While silicoflagellates are regularly seen in these waters in the spring and summer months (Ajani *et al.* 2001a), a bloom event of this magnitude had never previously been recorded in NSW waters (Hallegraeff 1991).

Class Dictyochophyceae (Silicoflagellates)

Silicoflagellates are unicellular cells with a single flagellum and a siliceous skeleton. Identification to species level is based on the shape of this silica skeleton. *Dictyocha* is the most common genus found in our waters and is perhaps toxic to fish (Box 6.7).

6.5.2 Prymnesiophyta=Haptophyta (Coccolithophorids, Prymnesiophytes)

Prymnesiophytes/haptophytes are unicellular or colony-forming flagellates that have two equal or unequal flagella, as well as a 'third flagellum' – a haptonema – a thin filamentous organelle sometimes used for anchoring the cell and sometimes in food uptake. Most species are small and belong to the nanoplankton (2–20 μm). The cell surface is covered with tiny scales or granules of organic material (cellulose), which is used extensively in taxonomy. In addition there may be spectacular calcified scales called coccoliths, which are characteristic of the coccolithophorids (Box 6.8). Coccolithophorids have formed geological deposits, such as the White Cliffs of Dover in the UK.

BOX 6.8 A COCCOLITHOPHORID BLOOM IN NSW
The NSW coastal embayment of Jervis Bay was once affected for four weeks by an unprecedented, mono-specific bloom of the small (<10 μm) cosmopolitan coccolithophorid *Gephyrocapsa oceanica* (Figure 6.3e). The bloom turned the waters milky green, which caused some economic hardship during the peak tourist season. Upwelling of cool nutrient-rich slope water and an influx of warm East Australian Current waters from an adjacent eddy may have enhanced the nutrients and upper layer temperatures and also the oceanic algal seed (Blackburn and Cresswell 1993). The maximum cell density of 2×10^7 cells/L (EPA unpublished) is greater than any previously recorded of this species in Australian waters.

6.5.3 Chryptophyta (Chryptomonads)

Chryptomonads are very small, ovoid phytoplankton (6–20 μm) with a rigid protein coat and two flagella protruding from a 'gullet' at one end (two equal or unequal in length, with one or two rows of tubular hairs).

6.5.4 Euglenophyceae

Euglenoids are large (15–500 μm), green, single-celled flagellates that have a deep fold or gullet where the flagellum is attached. The cell has a spiral construction and is surrounded by a pellicle that is composed of proteinaceous interlocking strips that wind helically around the cell (giving the cells a striped pattern). A conspicuous eyespot located in the cytoplasm can also usually be seen. Most of the Euglenophyta are freshwater species, with only a few marine species reported – mainly belonging to the genera *Eutreptiella*.

6.5.5 Chlorophyceae (Prasinophytes, Chlorophytes)

The chlorophytes (green flagellate algae) and the prasinophytes (scaly green flagellate algae) are the two main groups of the Chlorophyceae represented in coastal waters. The prasinophytes are generally small flagellates that are covered in organic scales. From one up to sixteen flagella (covered in minute scales and simple hairs) may be present and are used in many species to produce the characteristic stop and start swimming movement. The presence or absence and number of layers of scales covering the cell are used in the taxonomy of the group:

- scales absent (*Micromonas*)
- one layer of scales (*Mantoniella*)
- two or three layers (*Pyramimonas*)
- fused scales (*Tetraselmis*).

The chlorophytes represent a great variety of levels of organisation and include the macroalgae such as *Ulva, Enteromorpha, Cladophora* and *Caulerpa*. Marine microalgae are mainly represented by the genera *Dunaliella* and *Chlamydomonas*. These phytoplankton are distinguished by their bright green appearance, flagella and naked cell wall.

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Chapter 7

Freshwater zooplankton: diversity and biology

Tsuyoshi Kobayashi, Russell J. Shiel, Alison J. King and Anthony G. Miskiewicz

7.1 IDENTIFYING FRESHWATER ZOOPLANKTON

Zooplankton are present in most freshwater habitats, ranging from small temporary ponds to large permanent lakes. They are found in remote habitats such as lakes in the Antarctic (Bayly 1995) and near Mount Everest (Manca *et al*. 1994), and even in ground waters (Galassi 2001). Many species of freshwater zooplankton are small (less than 1 mm long) and relatively transparent. Exceptions to these are the larval stages of fish (see later discussion), some jellyfish that may reach 2–3 cm in diameter (Dumont 1994) and some Australian *Daphnia* that may reach 5–6 mm in the absence of predatory fish. Some alpine zooplankton may have bright red or other colours due to photo-protective pigments (Hessen and Sorensen 1990).

The important groups of freshwater zooplankton are larval fish, copepods, cladocerans, rotifers and protozoans. Larval fish in Australian freshwater systems can range in total length from approximately 2 to 20 mm, and can therefore be seen with the naked eye. Copepods and cladocerans are tiny crustaceans. Rotifers are distinctive little animals, with most species occurring only in freshwater. Protozoans are single-celled organisms and

Table 7.1. Typical freshwater zooplankton in Australia and elsewhere. Pelagic taxa are those occurring in open water (such as the centre of a lake or pond). Littoral taxa are those occurring among water plants near the shore or bank. The taxa marked by an asterisk * are illustrated in this chapter.

Pelagic copepods	Pelagic cladocerans	Pelagic rotifers
Calanoids: *Calamoecia** *Boeckella** *Eudiaptomus* *Diaptomus* *Gladioferens** Cyclopoids: *Australocyclops* *Cyclops* *Eucyclops* *Mesocyclops** *Metacyclops* *Thermocyclops* *Tropocyclops*	*Bosmina** *Ceriodaphnia** *Daphnia** *Diaphanosoma** *Moina* *Chydorus**	*Asplanchna** *Brachionus** *Conochilus* *Filinia** *Hexarthra* *Keratella** *Polyarthra* *Synchaeta* *Trichocerca**
Littoral copepods	**Littoral cladocerans**	**Littoral rotifers**
Calanoids: *Gladioferens** Cyclopoids: *Ectocyclops* *Eucyclops* *Macrocyclops* *Mesocyclops** *Paracyclops*	*Acroperus** *Alona* *Camptocercus* *Chydorus** *Ilyocryptus* *Macrothrix** *Neothrix* *Scapholeberis* *Simocephalus*	*Euchlanis* *Lecane* *Lepadella* *Notommata* Bdelloids

most are smaller than the other three groups. Rotifers and protozoans often go unnoticed, primarily because of their small sizes.

In freshwater, various prime habitats support different species (Table 7.1). Pelagic species are those occurring in open water (such as in the centre of a lake or pond) and are fully adapted to planktonic life. Littoral species are those occurring among water plants near the shore or bank. Littoral species are thus not truly planktonic, but constitute an important part of aquatic biota. The zooplankton in the littoral zone may be more species rich than those in the limnetic zone.

7.2 LARVAL FISH

Larval fish (or ichthyoplankton) are a common, seasonal and potentially diverse component of the zooplankton of the majority of freshwater habitats. Compared with estuarine and marine fish species, only a limited number of

identification guides are available for freshwater larvae (for example, Moser *et al.* 1984; Neira *et al.* 1998; Serafini and Humphries 2004).

Larval fish are often difficult to identify to the species level as they often have completely different morphological features to adults. As for estuarine fish, the most common method for identifying larvae is the series method or using existing keys and descriptions where they are available. The series method involves identifying the largest available larval or juvenile specimen, based or adult characteristics such as fin meristics and vertebral number (equivalent to the number of myomeres or muscle blocks – in larvae). The largest specimen is linked to smaller specimens in the series by using morphological and pigment characteristics. A variety of characters can be used to identify fish larvae including their general morphology such as the body shape and gut length and degree of coiling, the number of myomeres, pigmentation patterns (melanophores), the sequence of development of fins and the pattern of head spination (Table 7.2, Figure 7.1). The length and stage of development are important features in identification of larvae. For example the stage of flexion is when the notochord begins to grow upward (dorsally) and the bony structures of the tail fin begin to form on the ventral surface. Compared with the larvae of estuarine and marine fish, larvae of many freshwater species have a large yolk sac and morphological changes such as notochord flexion and development of the fin elements occurs at a larger size.

Most freshwater fishes have seasonal reproduction, with peaks in reproduction, and therefore larval abundance, generally occurring in spring and summer (Wooton 1998). Some species spawn over a relatively long time period (months), while others spawn period very briefly (a few days) (Matthews 1998). Therefore, the potential species composition of the ichthyoplankton is likely to change considerably from one sampling time to the next.

Larval fish can be found in rivers, creeks, lakes, reservoirs, off-channel habitats such as billabongs (ox bow lakes), wetlands and even in temporarily inundated habitats such as floodplains and ephemeral creeks. Larvae use a variety of habitat patches in freshwater systems, such as open water (pelagic) habitats, complex submerged macrophytes and woody debris, interstitial spaces of gravels, littoral habitats and backwaters. Some species also have fairly specific requirements at certain developmental stages; for example, many cyprinids have a downstream drifting dispersal phase, while other species require parental care in protected nest areas, such as in hollow logs.

The early life of fishes – from embryo to larvae to juveniles – is marked by rapid changes in morphology, ecology, growth and behaviour (Fuiman and Higgs 1997; Trippel and Chambers 1997). These changes often result in

Table 7.2. Freshwater fish larval characteristics (modified from Neira *et al.* 1998 and Serafini and Humphries 2004).

Family	Features
Eleotridae (gudgeon)	28–34 myomeres; body elongate; lightly pigmented; gut moderate and slightly coiled; conspicuous gas bladder; demersal eggs
Atherinidae (hardyhead)	34–36 myomeres; body very elongate; moderately pigmented; gut coiled and compact; demersal eggs
Cyprinidae (carp)	36–40 myomeres; body elongate; moderate to heavily pigmented; gut long and straight; demersal eggs
Gadopsidae (river blackfish)	49–50 myomeres; lightly pigment until late postflexion; large yolk sac; gut moderate to long and straight; demersal eggs
Galaxiidae (whitebait)	36–64 myomeres; body very elongate; lightly to heavily pigmented; gut long to very long and straight; demersal eggs
Melanotaenidae (rainbow fish)	34 myomeres; body elongate; moderately pigmented; gut short and coiled; demersal eggs
Retropinnidae (smelt)	45–53 myomeres; body very elongate; lightly pigmented; gut very long and straight; demersal eggs
Percichthyidae (cod/pigmy perch)	27–36 myomeres; body elongate to moderate; moderate to heavily pigmented; gut moderate to long and loosely coiled; large yolk sac in some genera; weak preopercular spines; demersal eggs
Plotosidae (catfish)	>77 myomeres; body elongate; moderately to heavily pigmented; gut moderate and loosely coiled; mouth barbells; large yolk sac; demersal eggs
Poeciliidae (*Gambusia*, mosquito fish)	31–33 myomeres; body moderate; moderately pigmented; gut short and coiled; live bearer
Percidae (redfin)	39–41 myomeres; body elongate; lightly pigmented; gut moderate and loosely coiled; conspicuous gas bladder; demersal eggs
Terapontidae (silver perch/ grunter)	25 myomeres; body elongate; lightly pigmented; gut coiled and moderate; small preopercular spines; demersal eggs

dramatic changes in habitat and diet use within a species. For example, some riverine fishes are exclusively found in shallow, still, off-channel habitats as newly hatched larvae, but then move to a variety of mid-channel habitats as older larvae and juveniles (see, for example, Scheimer and Spindler 1989). Similarly in lakes, many species occur in structurally dense, shallow, littoral habitats as small larvae and then move to mid water, deeper habitats as larger individuals. Movements of larval fish can also occur vertically, with diel migrations between surface waters and benthic habitats being common, particularly in deeper environments.

Larval fishes are a useful and sensitive tool for monitoring the effects of various anthropogenic influences on the system. For example, the presence of fish

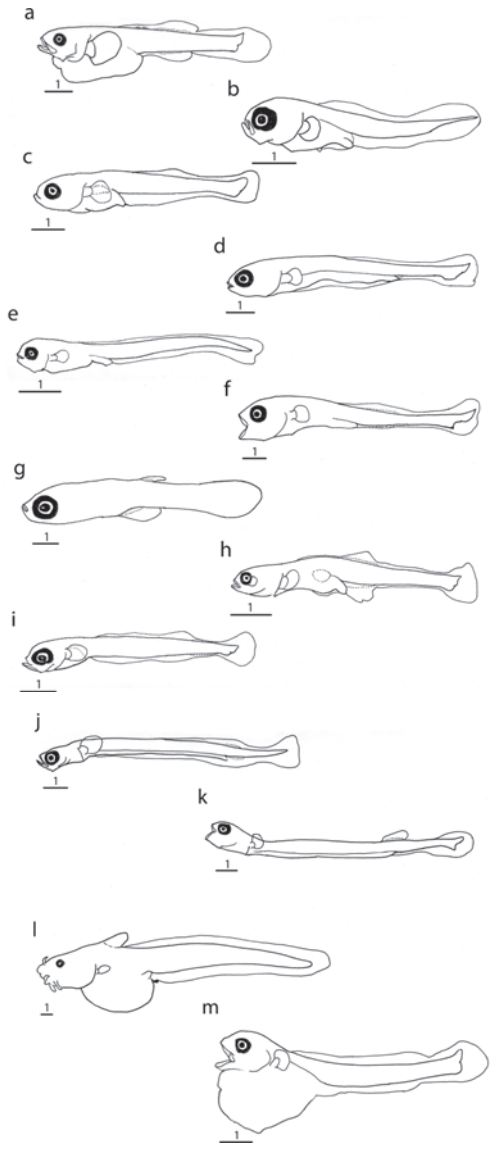


Figure 7.1 Outlines of larvae of some typical freshwater fish families approaching flexion. **a) Percichthyidae (cod), b) Percichthyidae (pigmy perch), c) Melanotaenidae (rainbow fish), d) Cyprindae (carp), e) Terapontidae (silver perch/grunter), f) Percidae (redfin), g) Poecliidae (*Gambusia,* mosquito fish), h) Eleotridae (gudgeon), i) Atherinidae (hardyhead), j) Galaxiidae (whitebait), k) Retropinnidae (smelt), l) Plotosidae (catfish), m) Gadopsidae (river blackfish). Scale bar is 1 mm. (Modified from Neira *et al.* 1998 and Serafini and Humphries 2004.)**

larvae clearly indicates that fish have spawned recently, and this can be used to elucidate the success of particular rehabilitation strategies targeted to enhance spawning, such as environmental flows (Humphries and Lake 2000).

7.3 COPEPODS

Freshwater planktonic copepods comprise two major groups: calanoids and cyclopoids. The calanoid copepods have an elongated body and long first antennae (Figure 7.2a), while the cyclopoid copepods have a stout body and

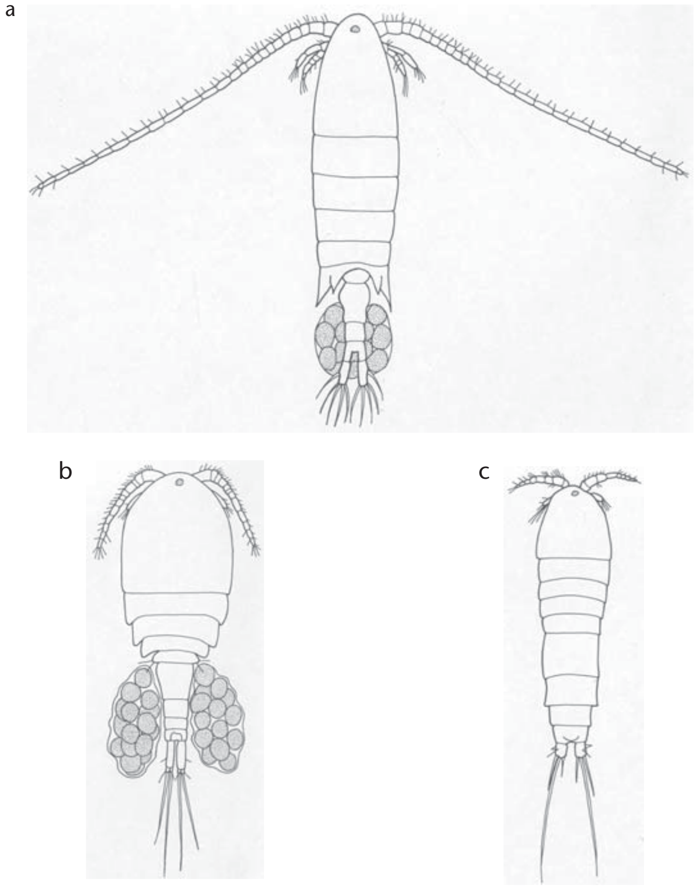


Figure 7.2 Three groups of freshwater copepods. a) Calanoid (egg-carrying female, dorsal view), b) cyclopoid (egg-carrying female, dorsal view), c) harpacticoid (female, dorsal view). (I. Faulkner.)

short first antennae (Figure 7.2b). A third group, the harpacticoids, have cylindrical bodies and very short first antennae. Harpacticoids are generally benthic, being found more often in or on the bottom mud or sand (Figure 7.2c). A key to the orders of freshwater copepods is shown in Table 7.3 (see also Figure 7.3).

The bodies of calanoids are often 1–2 mm long and cyclopoids and harpacticoids are usually less than 1 mm long. The body of a copepod is clearly segmented and females are larger than males. Females and males are also distinguished by the shape of the first antennae that are attached near the anterior end of the body and by other features (see Table 7.3 for details).

Copepods have pairs of different appendages on the ventral side of the body. For calanoid copepods, the appendages under the head are used for creating water currents to collect, filter and/or capture food particles. The appendages along the mid to lower body are used for swimming. Cyclopoid copepods use their mouth parts for capturing animal prey – most species

Table 7.3. Key to orders of freshwater copepods (Figure 7.3).

Phylum Subphylum Class	Arthropoda Crustacea Copepoda
1a First antennae long, slender body *Acanthodiaptomus, Calamoecia* (Figure 7.3a and 7.3b), *Boeckella* (Figure 7.3c), *Diaptomus, Eudiaptomus, Gladioferens* (Figure 7.3d), *Pseudodiaptomus* and others Key to sexes Right and left first antennae similar in shape = female Right and left first antennae dissimilar; right antenna geniculate (with an elbow-knee-like hinge) = male	**Order Calanoida**
1b First antennae short; head often much wider than lower body when seen from above *Australocyclops, Cyclops, Diacyclops, Macrocyclops, Mesocyclops* (Figure 7.3e) *Thermocyclops* and others Key to sexes Right and left first antennae similar in shape = female Right and left first antennae similar in shape, but geniculate and often strongly curved = male	**Order Cyclopoida**
1c First antennae short; cylindrical body *Canthocamptus, Fibulacamptus, Parastenocaris* (Figure 7.3f) and others Key to sexes Right and left first antennae similar in shape = female Right and left first antennae similar in shape, but geniculate = male	**Order Harpacticoida**

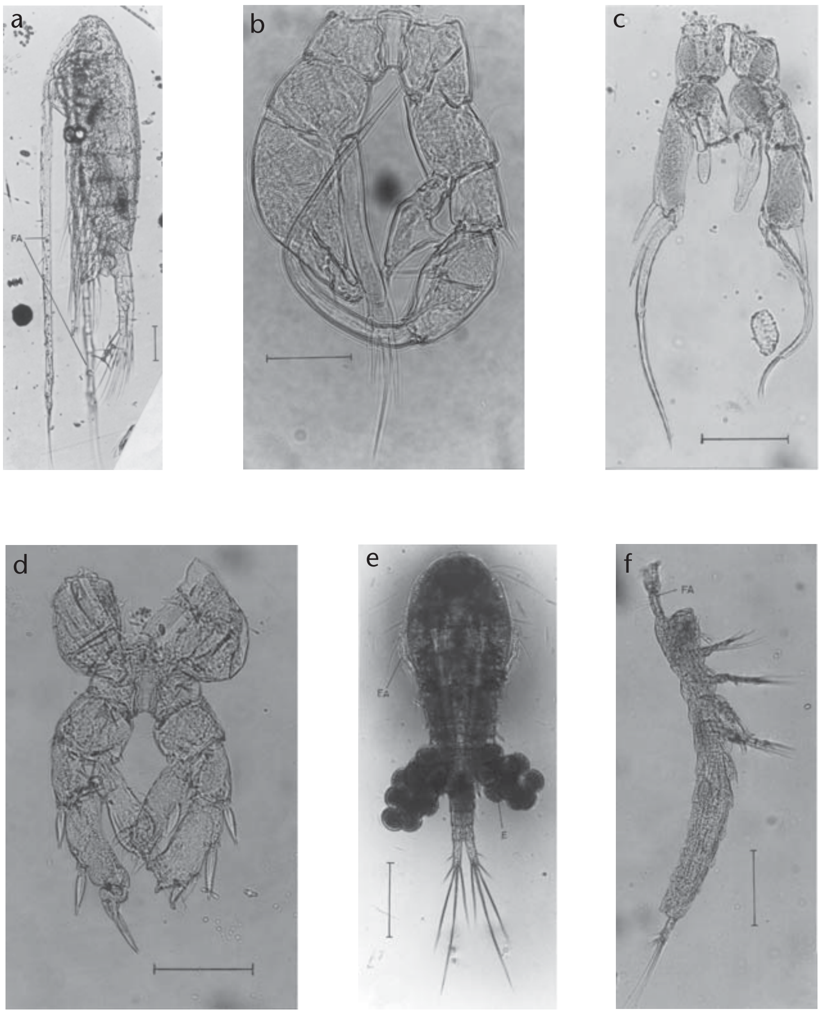


Figure 7.3 Copepods. a) *Calamoecia ampulla* – Body elongated, with long first antennae (FA). Small calanoid copepod. Male fifth legs need to be examined for identification of species. Scale bar 100 µm; b) *Calamoecia ampulla* – Male fifth legs (posterior aspect). Scale bar 50 µm; c) *Boeckella fluvialis* – Male fifth legs (posterior aspect). Body elongated, with long first antennae. Relatively large calanoid copepod. Scale bar 100 µm; d) *Gladioferens pectinatus* – Male fifth legs (anterior aspect). Body elongated, with long first antennae. Relatively large calanoid copepod in fresh and salt water. Scale bar 100 µm; e) *Mesocyclops* sp. – Body relatively stout, with short first antennae (FA). Scale bar 200 µm; f) *Parastenocaris* sp. – Body cylindrical, with very short first antennae (FA).Bottom dwelling, but may also appear in plankton.

are carnivorous. The legs along the mid to posterior body of copepods are mainly used for swimming. Calanoids and cyclopoids have five pairs of swimming legs and harpacticoids have five or six pairs. The detailed structure of fifth legs in the male is useful in identifying calanoid species. Fourth and fifth legs in the female are important in identifying cyclopoid species. All swimming legs are important in identifying harpacticoid species.

Copepods moult up to 11 times before becoming adults, with body shape and size changing after each moult. There are two distinct young stages: nauplius larvae and copepodites. A nauplius larva looks very different from an adult. A copepodite has fewer body segments and appendages, but looks like a small adult.

Female copepods produce eggs that always need to be fertilised by males. Females carry the eggs in one or two sacs attached to the ventral side of the body. The egg sacs and eggs are easily observed under a microscope. Some copepods produce resting eggs that withstand drought and other adverse environmental conditions. One study reported that the resting eggs of certain calanoid copepods can live in lake sediments for as long as 400 years (Hairston *et al*. 1995)!

Calanoids eat a wide variety of phytoplankton species and other suspended matter such as decayed plant material and clay particles. Some eat other small zooplankton, such as rotifers and ciliated protozoans. Cyclopoids are primarily carnivorous – eating other zooplankton.

Copepods may occur in the plankton all year round, usually reaching densities of 5–20 animals per litre in ponds, lakes, reservoirs and slow-flowing rivers.

7.4 CLADOCERANS

Most cladocerans are less than 1–2 mm long, but there are some notable exceptions: specimens 5–6 mm in length have been found in some water bodies. Females are usually larger than males. The body consists of a rigid, clam-like shell – called a carapace – which is transparent, but can be yellowish or brownish in colour. Pairs of appendages called thoracic limbs are inside the carapace and are important for collecting and transferring food particles to the mouth. The head of a cladoceran is usually compact, with prominent eyes and large antennae used for swimming. Some cladocerans develop conspicuous head and tail spines, helmet or 'neck-teeth' (Figure 7.4). A key to the families of freshwater cladocerans is shown in Table 7.4 (see also Figure 7.5). Cladoceran taxonomy is constantly being reviewed and it is likely that additions of new families will occur (e.g. Santos-Flores and Dodson 2003).

Table 7.4. Key to families of freshwater cladocerans (modified from Smirnov and Timms 1983) (Figure 7.5).

Phylum	Arthropoda	
Subphylum	Crustacea	
Class	Branchiopoda	
Order	Diplostraca	
Suborder	Cladocera	
1a Body and swimming legs not covered with a carapace		→ 2
1b Body and swimming legs covered with a carapace		→ 3
2a Body short with four pairs of swimming legs Family Polyphemidae: *Polyphemus* 2b Body long with six pairs of swimming legs Family Leptrodridae: *Leptodora*		
3a Six pairs of swimming legs inside the carapace all similar		→ 4
3b Five or six pairs of swimming legs inside the carapace not similar		→ 5
4 Body length much greater than body height; second antennae with large branch-like appendages Family Sididae: *Diaphanosoma* (Figure 7.5e) and others		
5a First antennae long and slender, like an elephant's trunk Family Bosminidae: *Bosmina* (Figure 7.5a) and *Bosminopsis*		
5b First antennae usually short		→ 6
6a Second antennae two-branched, both with three segments; mostly small body length, hemispherical or circular in lateral view Family Chydoridae: *Acroperus* (Figure 7.5b), *Alona, Chydorus* (Figure 7.5d), *Graptoleberis, Pleuroxus* and others		
6b Second antennae two-branched, one with three segments and the other with four segments		→ 7
7a First antennae not flexible and short Family Daphniidae: *Ceriodaphnia* (Figure 7.5c), *Daphnia* (Figures 7.4 and 7.6), *Simocephalus* and others		
7b First antennae flexible and long relative to body length		→ 8
8a First antennae on mid-abdominal side of head; oval body Family Moinidae: *Moina* and *Moinodaphnia*		
8b First antennae on frontal side of head		→ 9
9a Postabdomen with distal, terminal claw Family Macrotrichidae: *Macrothrix* (Figure 7.5f) and others 9b Postabdomen lacks terminal claw Family Neotrichidae: *Neothrix*		

Female-only populations of cladocerans occur under normal environmental conditions. They produce female eggs inside a chamber on the dorsal side of the body, within which the eggs hatch. Newly hatched young – which look like small adults – remain there until they are ready to swim.

When environmental conditions deteriorate (through a lack of food or drying of the water body), the females produce eggs that hatch into males. Fertilised females then produce one or two special resting eggs encased in a thick protective covering to form an ephippium, which is released into the



Figure 7.4 A species such as *Daphnia lumholtzi* can produce conspicuously long head and tail spines, resulting in the extension of an overall body length. Long head and tail spines can make it more difficult for fish to eat *Daphnia,* thus reducing the level of predation by fish.

water (Figure 7.6). Ephippia can withstand a wide range of environmental conditions, surviving for many years in dry sediments. Cladocerans can establish new populations from ephippia when environmental conditions once again become favourable.

Cladocerans moult several times as they grow to adulthood. A new carapace is formed inside the old, which is then discarded as the body grows bigger. The discarded carapaces are called exuviae. Collections of plankton samples may contain exuviae as well as live animals. Exuviae can also be used to identify species that have occupied a habitat in the past. Those preserved in sediments can also be used to identify past occupants of habitats up to 10 000 years ago. The science of studying such remains is called palaeolimnology, and is helpful in understanding past environmental conditions and climate change.

Cladocerans, especially large *Daphnia*, eat a wide variety of phytoplankton and other suspended matter, such as decayed plant material and clay particles. They may greatly reduce phytoplankton abundance. There are several genera of carnivorous cladocerans.

Cladocerans occur normally from spring to early summer, reaching densities of 10–30 animals per litre in ponds, lakes and reservoirs. In a special

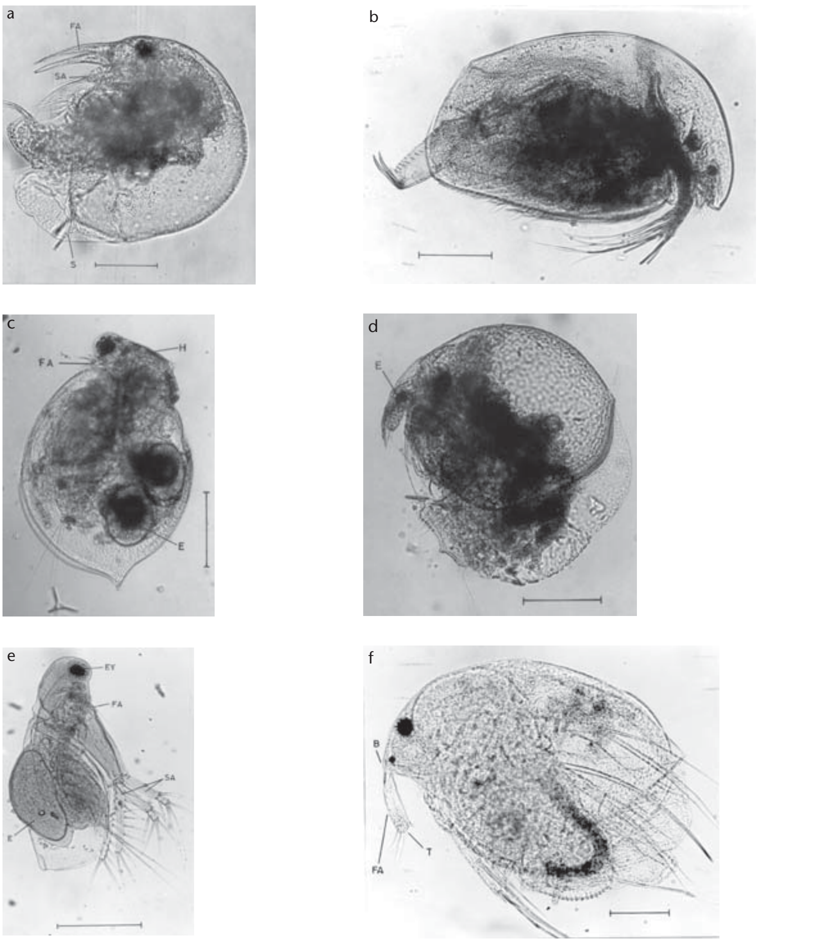


Figure 7.5 Cladocerans. a) *Bosmina meridionalis* – Small body. First antennae (FA) relatively long, slender, not fused at their bases. Second antennae (SA) relatively small. Often a pair of spine-like elongation (S) at ventro-posterior corner of body. Scale bar 100 μm; b) *Acroperus* sp. – Body flattened laterally. Bottom dwelling. Normally found among water plants. Scale bar 100 μm; c) *Ceriodaphnia* sp. – Body shape broadly oval. Head (H) small. Short first antennae (FA). Normal eggs (E). Scale bar 200 μm; d) *Chydorus* sp. – Body small, spherical. Small eyes (E). Bottom dwelling, but also appears in plankton. Scale bar 100 μm; e) *Diaphanosoma excisum* – Body without a tail spine. Head relatively large, rectangular with large eye (EY). First antennae (FA) small. Second antennae (SA) large and well developed. Large normal egg (E). Scale bar 300 μm; f) *Macrothrix spinosa* – Body flattened laterally, without tail spine. First antennae (FA) situated frontal side of head. Tip of first antennae (T) wider than its base (B). Bottom dwelling. Normally found among water plants. Scale bar 100 μm.

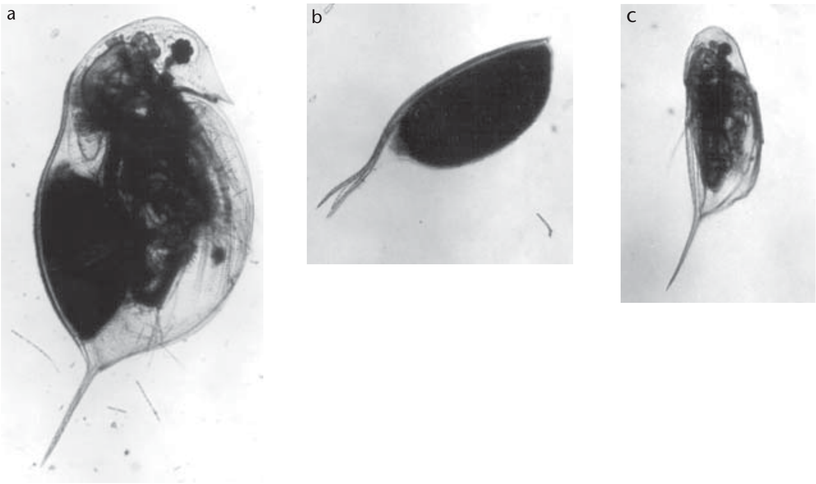


Figure 7.6 ***Daphnia's*** **resting eggs in an ephippium can survive in adverse environmental conditions, even after the females that produced the ephippium die. a) An ephippium is formed on the dorsal side of a female, b) the ephippium usually detaches after the female dies, c) young** ***Daphnia*** **will hatch from the resting eggs when the environmental conditions become favourable again.**

case, a high density of 500 cladocerans per litre has been reported from a waste stabilisation pond (Mitchell and Williams 1982).

7.5 ROTIFERS

Most rotifers are 0.1–0.5 mm long. Their body shape varies widely between groups: they can be spherical, cylindrical or elongated. The body can be soft or may have a firm covering called a lorica. Some rotifers are enclosed in a gelatinous case. Many have different types of spines and a foot. Some even have toes. The structure of the jaw (or trophi) is distinctive for each species and is used for identification (it is necessary to dissolve body tissues with a chemical, such as bleach, to observe the jaws). The cilia surrounding a rotifer's mouth form a circle, called a corona or wheel organ. The rapid movements of the cilia create water currents for swimming and feeding. A key to the orders and families of freshwater rotifers is shown in Table 7.5 (see also Figure 7.7).

Rotifer populations consist only of females under normal environmental conditions. They produce eggs that hatch into females without the need for male fertilisation (a process known as parthenogenesis). The eggs are

Table 7.5. Key to orders of freshwater rotifers (modified from Shiel 1995) (Figure 7.7).

Phylum Class	Rotifera Monogononta/Bdelloidea
1a Body with a single ovary; body often with a lorica or tube	**Class Monogononta 2**
1b Body with paired ovary; body without a lorica or tube	**Class Bdelloidea**
Orders Adinetidae, Philodinidae Philodinavidae (fresh to brackish) and others	
2a Mastax malleoramate	**Order Flosculariacea**
Family Conochilidae: *Conochilopsis* and *Conochilus* Family Flosculariidae: *Floscularia, Lacinularia, Sinantherina* and others Family Testudinellidae: *Pompholyx, Testudinella* and others Family Trochosphaeridae: *Filinia* (Figure 7.7d) and others	
2b Mastax not malleoramate	⟶ 3
3a Mastax uncinate	**Order Collothecacea**
Family Collothecidae: *Collotheca* and others	
3b Mastax not uncinate	**Order Ploima**
Family Asplanchnidae: *Asplanchna* (Figure 7.7a) and others Family Brachionidae: *Anuraeopsis, Brachionus* (Figure 7.7b), *Keratella* (Figure 7.7e), *Notholca, Platyias* and others Family Gastropodidae: *Ascomorpha* and *Gastropus* Family Lecanidae: *Lecane* Family Lepadellidae: *Colurella, Lepadella* and *Squatinella* Family Mytilinidae: *Mytilina* Family Notommatidae: *Cephalodella* (Figure 7.7c), *Monommata* and others Family Synchaetidae: *Polyarthra, Synchaeta* and others Family Trichocercidae: *Ascomorphella, Elosa* and *Trichocerca* (Figure 7.7f) Family Trichotriidae: *Trichotria* (Figure 7.7g) and others	

relatively large compared to the body size of females, and are normally attached to the posterior part of their bodies before being released in water. It may take less than a week for juveniles of many rotifers to become mature.

However, under certain conditions, females produce eggs that hatch into males. Fertilised female rotifers then produce special resting eggs. The resting eggs can withstand extreme temperatures, drought and other adverse conditions. The eggs can remain viable long after the female rotifers that produced them have died. The resting eggs remain dormant – buried in the sediments for many years. New populations of female rotifers can establish from resting eggs when environmental conditions become favourable.

Rotifers eat bacteria, including cyanobacteria, and phytoplankton. Some are carnivorous and eat other rotifers. Rotifers may be abundant in

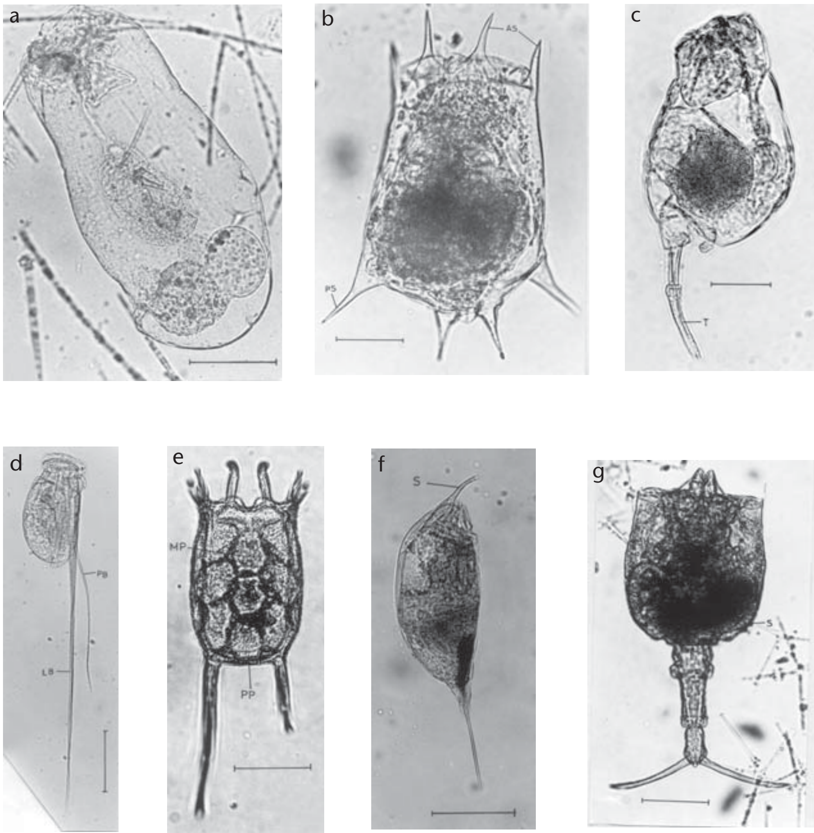


Figure 7.7 Rotifers. **a)** ***Asplanchna priodonta*** **– Foot absent. Body transparent. Specimen preserved in formalin often strongly contracts. Jaw (trophi) needs to be examined for identification of species. Scale bar 100 µm; b)** ***Brachionus calyciflorus amphiceros*** **– Four anterior spines (AS) on dorsal side of lorica. Long posterior spines (PS). Scale bar 50 µm; c)** ***Cephalodella gibba*** **– Body fusiform, with slender toes (T); d)** ***Filinia longiseta*** **– Body shape oval. Body with two long lateral bristles (LB) and one short posterior bristle (PB). Scale bar 100 µm; e)** ***Keratella tropica*** **– Three six-sided median plaques (MP) on dorsal side of lorica. Single small four-sided posterior plaque (PP). Scale bar 50 µm; f)** ***Trichocerca chattoni*** **– Body cylindrical, more or less squat. Single long curved spine (S) at margin of head opening. Scale bar 100 µm; g)** ***Trichotria*** **sp. – Head, body and foot segments distinctive and rigid. Lorica margin with small spines (S). Scale bar 50 µm.**

both standing and running waters. A maximum of 3500 rotifers have been recorded from one litre of water in an Australian river (Kobayashi *et al.* 1998). It is common to find more than 20 000 rotifers per litre in some billabongs and also in some reservoirs.

7.6 PROTOZOANS

Protozoans are generally microscopic (much less than 1 mm long). They have various body shapes (spherical, oval or elongate) and often have one or more long, fine, whip-like appendages, called flagellae, or many short hair-like structures, called cilia. Some produce temporary foot-like protrusions called pseudopodia. These body parts are important for locomotion and feeding. A key to the phyla of protozoans is shown in Table 7.6 (see also Figure 7.8).

Protozoans eat bacteria, including cyanobacteria, and small phytoplankton. Some are carnivorous and eat other zooplankton (for example, the ciliate *Bursaria* may include rotifers in their diet). Protozoans grow quickly and increase in numbers by means of cell duplication. They are abundant in many types of water bodies, from fish tanks and sewage ponds to lakes and reservoirs. In running waters, such as streams and rivers, protozoans found in the plankton are often those that have been swept from the surfaces of submerged rocks, water plants or sediments.

7.7 SPECIFIC ISSUES IN SAMPLING AND MONITORING

Temporal and spatial scales of zooplankton sampling and monitoring in fresh water depend on the type and extent of ecological concern, issues and hypotheses that are going to be put forward and tested. The general

Table 7.6. Key to phyla of protozoans (modified from Jahn *et al.* 1979) (Figure 7.8).

1a Body with cilia or tentacles	Phylum Ciliophora (often called ciliates)
Epistylis (Figure 7.8c), *Frontonia, Paramecium, Paradileptus* (Figure 7.8e), *Vorticella* and others	
1b Body without cilia or tentacles	→ 2
2a Body with other structures for locomotion	→ 3
2b Body without obvious structures for locomotion	→ 4
3a Body with one or more flagella	Phylum Mastigophora (often called flagellates)
Ceratium, Euglena, Peridinium and others	
3b Body with pseudopodia	Phylum Sarcodina (often called amoebae)
Arcella (Figure 7.8a), *Cyphoderia* (Figure 7.8b), *Euglypha* (Figure 7.8d), *Difflugia,* amoebae without a rigid test (Figure 7.8f) and others	
4 Movement by body flexions; all parasitic	Phylum Sporozoa
Plasmodium (the causative organism of malaria) and others	

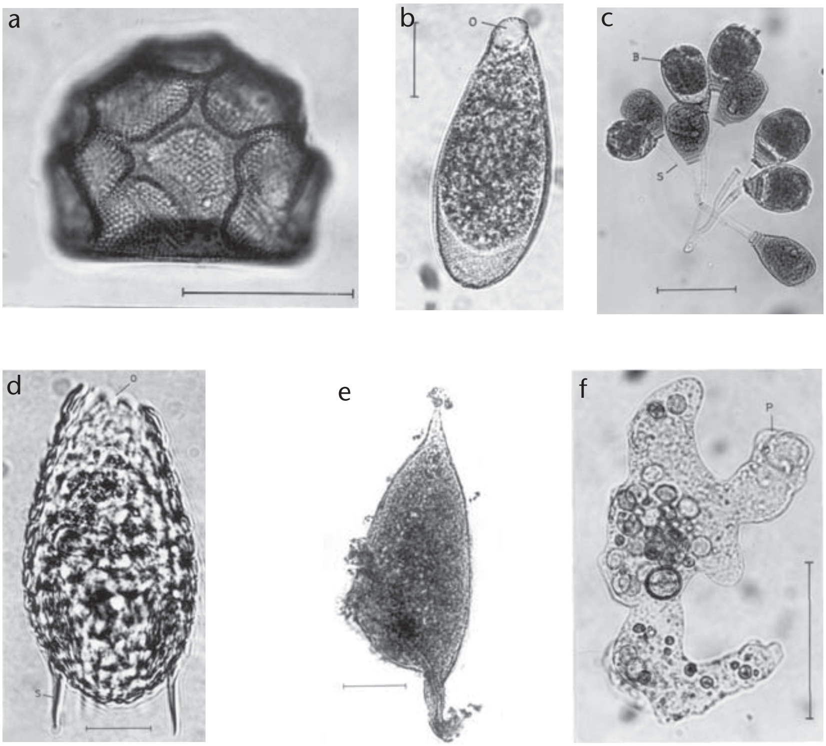


Figure 7.8 Protozoans. a) *Arcella mitrata* – Body with test. Test circular from above, dome-like on top. Small central opening. Scale bar 50 µm; b) *Cyphoderia* sp. – Body with test. Test oval, short cylindrical neck. Round opening (O) oblique to body of test. Test with a yellow-brown matrix. Scale bar 30 µm; c) *Epistylis* sp. – Bell-shaped body (B), with a stalk (S). Stalk splits into two branches and cannot contract. Scale bar 100 µm; d) *Euglypha* sp. – Body with oval test, made of scales of equal sizes. Opening (O) terminal. Some with spines (S) on test. Scale bar 20 µm; e) *Paradileptus* sp. – Body with cilia. Relatively large protozoans. Scale bar 50 µm; f) Amoeba (unidentified) – Body with no test and no cilia. Note pseudopodia (P). Scale bar 100 µm.

framework of ecological sampling and monitoring and statistical considerations are applicable to zooplankton sampling and monitoring (such as the original BACI design or its modifications and trend analyses). A pilot sampling and monitoring program is always helpful in determining the methods of sampling (for example, plankton net versus plankton trap) and in providing basic data on species composition, density, biomass and their variability.

There is a large diversity of types of gear currently available for the collection of larval fish in freshwater habitats. The most commonly used types

of gear are designed to filter volumes of water through fine mesh, including drift nets, trawl nets, seines and pumps with fitted mesh nets (Kelso and Rutherford 1996). Electrofishing gear modified for sampling small-bodied fish has also recently been used increasingly in freshwater habitats (Copp 1989; King and Crook 2002). There are also a range of more passive collection gears, such as light traps, baited traps and activity traps – where fish are either attracted into the trap or are captured while moving through the habitat. However, knowledge of the target fish reproductive life history and larval behaviour and ecology is required in the choice of collection methods, gear types, sampling periodicity and sampling habitat.

For other types of zooplankton, a conical plankton net is often useful in collecting pelagic species (Table 7.7). Depending on the mesh size and specifications of the plankton net used, the net may clog partially or fully after towing certain distances and its filtering efficiency may drop dramatically. The clogging of a net is primarily due to collection of phytoplankton and detrital particles that are larger than the mesh size. This problem is often encountered in eutrophic waters as well as highly turbid waters. The volume of water filtered by the net needs to be calibrated with a flow meter if zooplankton are need to be collected quantitatively (see Chapter 4).

Zooplankton are seldom distributed uniformly within a water body. Some species exhibit a diurnal vertical migration – often concentrating in deep waters during the day and in surface waters during the night (see Chapter 2). Zooplankton samples should be collected in a depth-integrated manner from the bottom to the surface or from multiple discrete depths.

It is difficult to properly tow a plankton net in the littoral zone – often resulting in the collection of large amounts of aquatic-plant debris that clog the net. Specialised sampling devices and techniques are recommended to use in collecting littoral zooplankton (Campbell *et al*. 1982; Sakuma *et al*. 2002).

7.8 CONCLUSIONS

Zooplankton are diverse and ubiquitous organisms in fresh water. Zooplankton occupy an intermediate trophic level – functioning as an important food source for a variety of animals, including juvenile and larger fish. In turn, they can be important in the control of bacterial and algal abundances and quickly increase in number following increased bacterial and algal numbers.

Zooplankton are also sensitive to various substances that enrich or pollute water, and have often been used as indicators to monitor and assess the condition and change of the freshwater environment, particularly in

Table 7.7. Sampling devices for freshwater zooplankton.

Type	Comments	References
Conical or cylindrical-conical plankton nets	Widely used, different type of nets available, easy to deploy, very suitable for depth-integrated as well as horizontally integrated samples. The filtration efficiency of a net must be determined for more quantitative sampling of zooplankton.	Evans and Sell (1985), Wetzel and Likens (1991), McQueen and Yan (1993)
Bottles (e.g. Van Dorn and Niskin samplers)	Suitable for fixed volume sampling and discrete depth sampling. Light weight allowing samples to be taken easily from a small boat. Effective in collecting small organisms such as protozoans and rotifers.	Eaton *et al*. (2005)
Traps (e.g. Schindler-Patalas trap)	Suitable for fixed volume sampling, and discrete depth sampling. Light weight allowing samples to be taken easily from a small boat. Suitable for collecting larger organisms, such as adult copepods and cladocerans, as well as small rotifers and protozoans.	Schindler (1969), Haney (1971), Shiel *et al*. (1982), Wetzel and Likens (1991)
Pumps	Easy to deploy; suitable for collecting littoral organisms, such from the surface of submerged aquatic plants.	Campbell *et al*. (1982), Malone and McQueen (1983), Sollberger and Paulson (1992)

the northern hemisphere (see Chapter 3.6). They display fairly consistent, measurable changes to water quality and various forms of pollution. These findings provide a basis for 'where to look' when zooplankton are used as indicators in freshwater ecosystems.

As a general trend, microzooplankton are more tolerant than macrozooplankton to different forms of pollution. Possible mechanisms to explain this trend include:

- reduced food availability for large zooplankton in acidified systems (Havens 1991)

- the short generation time and ability to recover quickly after stress shown by small zooplankton in agricultural pollution (Havens and Hanazato 1993)
- the predation of large zooplankton (particularly *Daphnia*) by fish in eutrophication (Brooks and Dodson 1965).

Zooplankton have been frequently used as ecotoxicological test organisms to assess the acute and chronic effects of various toxic substances that are found in the freshwater environment. Importantly, the lethal and effective values obtained from these bioassays are not necessarily applied to the evaluation of ecosystem impact of a toxicant. For example, Lampert *et al*. (1989) reported that *Daphnia* showed low sensitivity to the herbicide atrazine when direct effects (that is, acute toxicity) were measured, but became very sensitive to the chemical in the moderately complex 'food chain' mesocosm experiment. Clearly, biological interactions play a significant, and unexpected role in the modified response of *Daphnia*.

Pollution management and monitoring programs that depend on a small number of indicators may fail to consider the full complexity of ecosystems. It may be necessary to use a suite of indicators representative of the structure, function and composition of ecosystems (Dale and Beyeler 2001). The useful application of zooplankton as indicators in freshwater ecosystems can only be realised by understanding the characteristics and dynamics of the ecosystems that are subject to various water resource management activities. In addition, the design of any monitoring program needs to consider the importance of temporal and spatial variability in sampling for zooplankton, to allow for meaningful conclusions from the data.

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Chapter 8

Coastal and marine zooplankton: diversity and biology

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8.1 IDENTIFYING MARINE ZOOPLANKTON

Fresh zooplankton – even freshly preserved and rinsed – is quite amazing to look at under the microscope, but to the naked eye the sample may seem a little disappointing after the anticipation of towing a net for 10 minutes. Remove the sticks and large jellyfish (thoroughly rinse off formalin using a fine sieve if necessary), sit down at a comfortable and well set-up microscope and enjoy the complexity, diversity and colours of these fascinating creatures. Try drawing some simple sketches of dominant types to focus your attention onto the basics of identification outlined below.

Within a sample of marine zooplankton, you may find the adults or larvae of nearly all of the Earth's living phyla, although it will usually be dominated by the crustaceans – mostly copepods (Figures 8.1–8.3). Like any arthropod (invertebrates with an exoskeleton), copepods grow by shedding their exoskeleton through a series of moults (or instars, or developmental stages), so that the diversity of shapes is potentially 10 fold greater than the number of species! You may also find drowned insects or a few rare marine insects or mites.

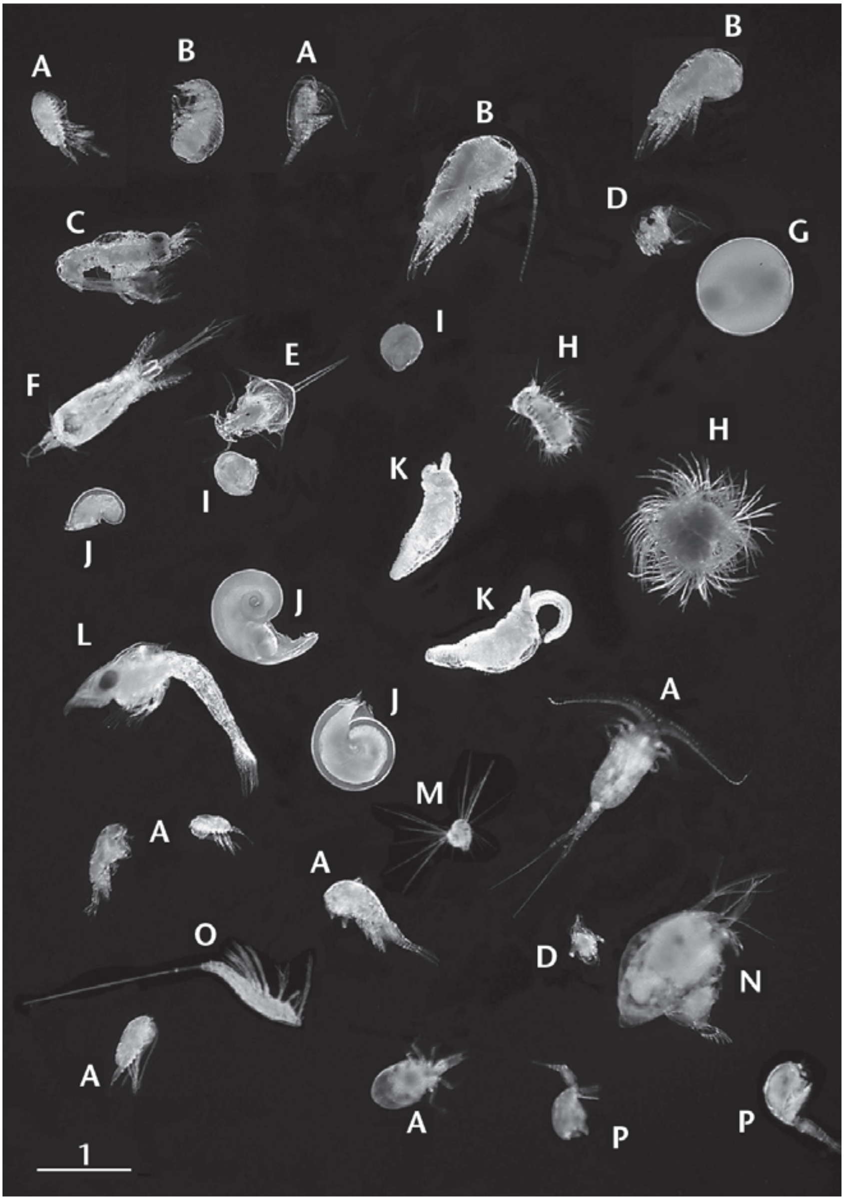


Figure 8.1 a. Smaller zooplankton (~ 1 mm across) showing A–calanoid and cyclopoid copepods, B–hyperid amphipods, C–larval prawn, D–cladocerans, E–crab zoea, F–cyclopoid copepod, G–an invertebrate egg, H–larval polychaete worms, I–bivalve, J–pteropods, K–polychaete larvae, L–larval decapod (anomuran), M–early stage juvenile polychaete, N–ostracod, O–harpacticoid copepod, P–juvenile copepods or copepodites.

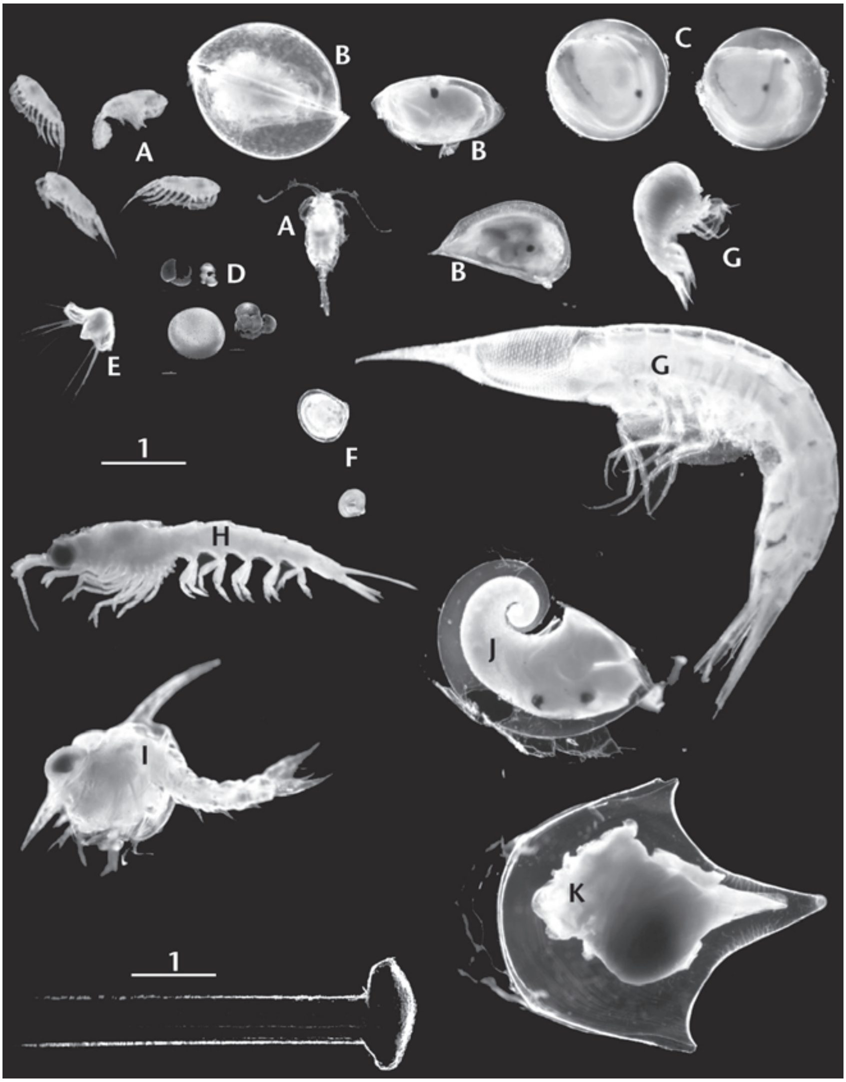


Figure 8.1 b. Smaller zooplankton caught off eastern Australia, with reference to the size of a pin (width of pin is 0.6 mm), showing A–copepods, B–ostracods, C–fish eggs, D–globigerinid shells, E–juvenile polychaete worm, F–bivalves, G–hyperid amphipods, H–juvenile krill, I–crab zoea, J–planktonic snail, a heteropod, *Atlanta,* K–planktonic snail, a pteropod.

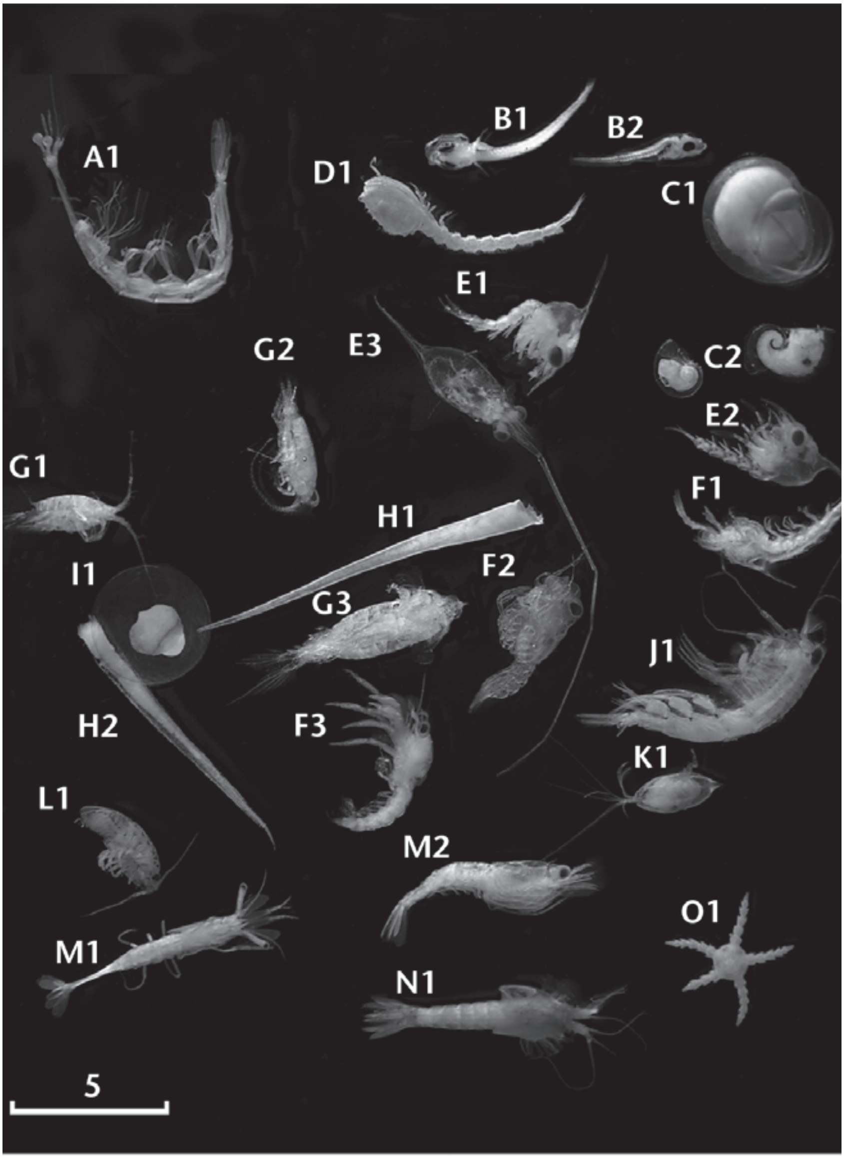


Figure 8.2 a. Medium-sized zooplankton caught off eastern Australia showing A–sergestid or ghost shrimp *Lucifer,* B–larval fish, C–planktonic snails, D–cumacean, E–larval crabs (zoeae), F–later stage crab larvae (megalopae), G–copepods, H–pteropods, I–fish egg, J–gamarid amphipod, K–ostracod, L–isopod, M–juvenile prawn or carid shrimps, N–mysid, O–brittle starfish.



Figure 8.2 b. Medium-sized zooplankton (width of pin is 0.6 mm) showing A–calanoid copepods, B–isopods, C–gammarid amphipods, D–late stage crab larva (megalopa), E–larval crab (zoea stage), F–mysids, G–heteropods, *Atlanta,* H–juvenile shrimp, I–larval prawn with tail oriented upwards, J–larvaceans, K–calanoid copepods, *Gladioferens,* L–cladocerans *Podon,* M–salp or doliolid, N–larval fish, goby, O–cnidarian, jellyfish, P–pteropods, Q–polychaete, R–mysids, note the distinctive balance organs or statocysts within the tail-fan.

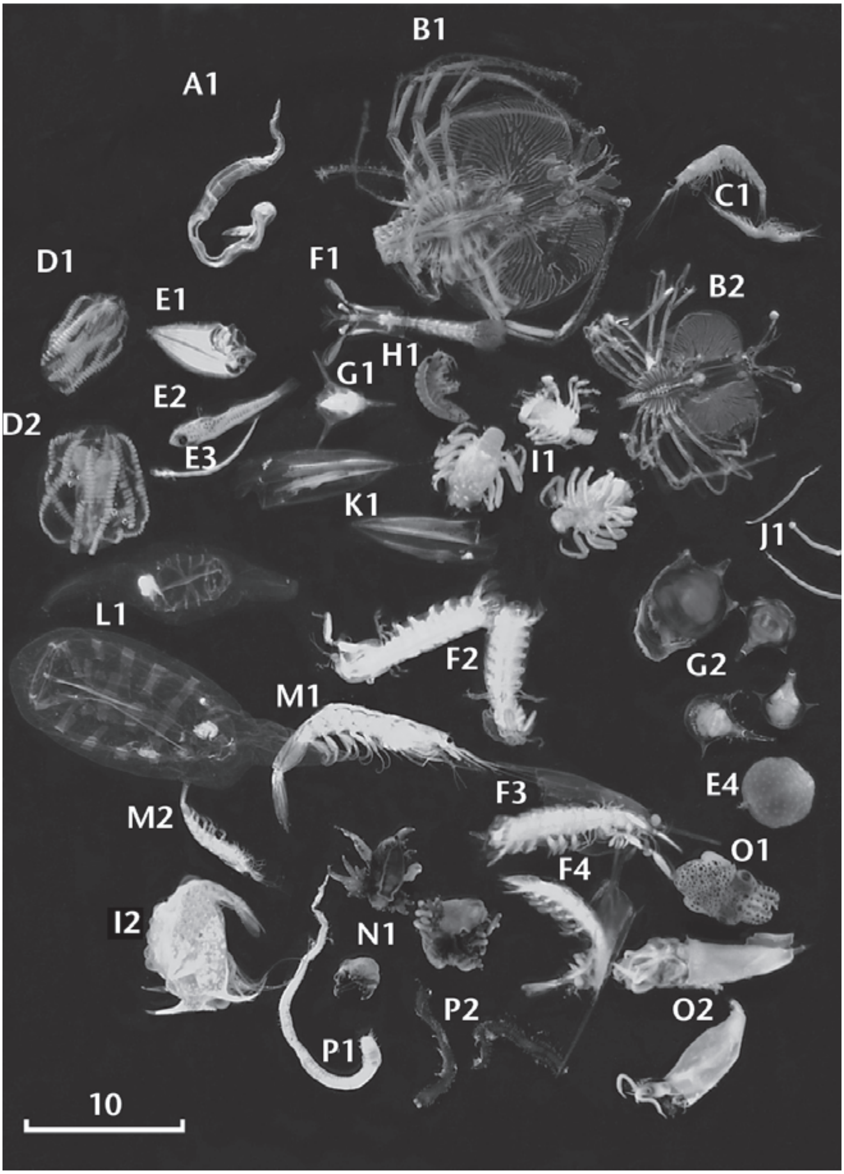


Figure 8.3 a. Larger-sized zooplankton caught off eastern Australia showing A–chaetognath, B–larval lobsters (puerulus stage), C–juvenile prawns, D–ctenophore, E–larval fish including flatfish, herring, goatfish, F–stomatopod zoea, G–pteropods, H–amphipod, I–late stage crab larvae (megalopae), J–smaller chaetognaths, K–siphononophore, L–salps, M–juvenile prawns, N–three small, *Glaucus* (a bright blue sea slug), O–larval squid and octopus, P–polychaetes.

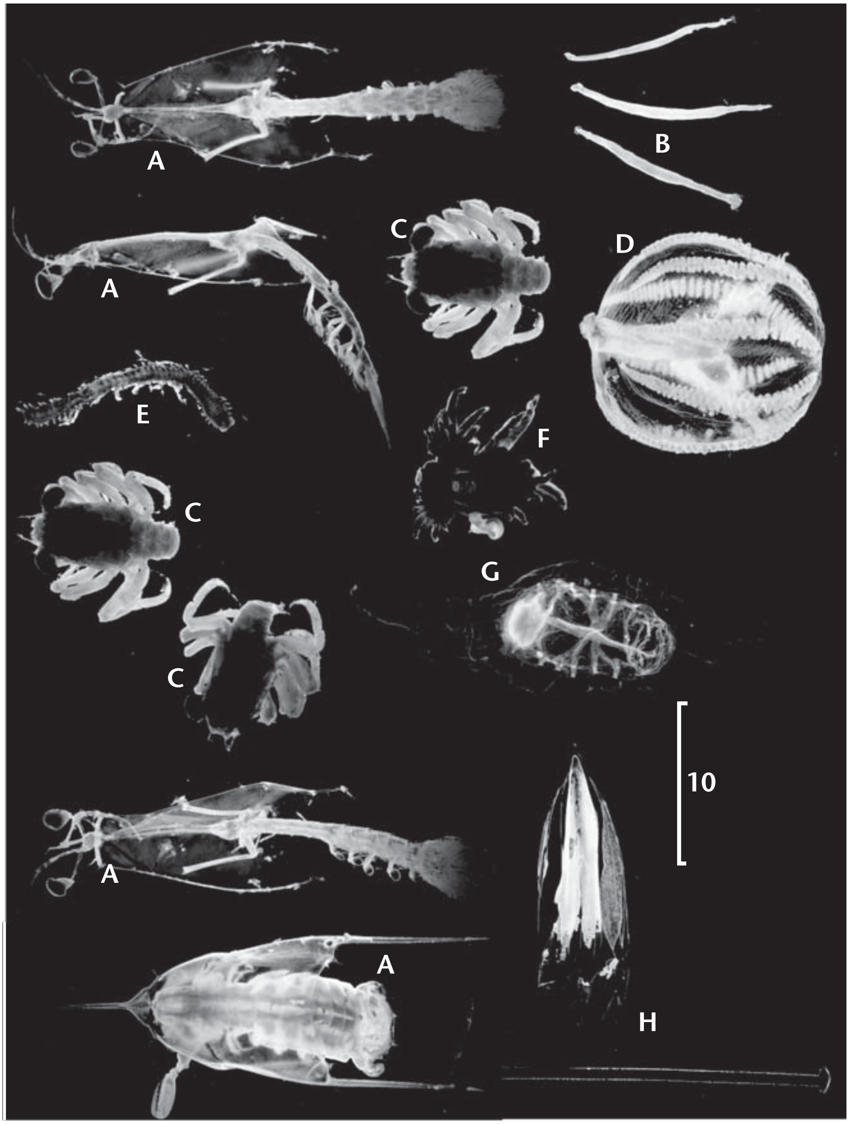


Figure 8.3 b. Larger-sized zooplankton a with reference to the size of a pin (width of pin is 0.6 mm), A–late stage larval stomatopods, B–chaetognaths, C–late stage crab larvae (megalopae), D–tentaculate ctenophore (lobate ctenophores are too delicate to capture whole), E–polychaete, F–pelagic sea slug *Glaucus,* G–salp, H–siphonophore.

A typical sample is shown that has been sorted into small (<1 mm, Figure 8.1), medium (1–3 mm, Figure 8.2) and large (>5 mm, Figure 8.3). Our minds are good at recognising characteristic shapes, so at an initial level, no dichotomous keys are necessary. Shape and body size – as indicated by the approximate scale bar – are the two essential aspects of identifying zooplankton in different orientations. The scale bar is only approximate as the actual size can vary with respect to the latitude (temperature) or rearing conditions in the laboratory.

Crustaceans are the first things we recognise – by their eyes and many limbs (Figures 8.1–8.3). The eyes are either stalked and obvious, or are sessile and compound (that is, eyes that look rather like dabs of black paint on the exoskeleton). A compound eye is made up of many elements, rather like pixels. Another useful distinction is the presence or absence of a carapace or shell that covers their main walking (thoracic) limbs and gills. For example, most prawn-like crustaceans have a carapace, but brine shrimps, copepods, amphipods and isopods do not. Some small crustaceans are enclosed by their carapace (cladocerans and ostracods). The generalised body plan of a crustacean is well illustrated by a lobster – with a head, a thorax covered by the carapace and a long abdomen. You will find the number and location of limbs on the three body sections to be a useful characteristic. Crustaceans have two pairs of antennae on the head and (like every other limb) are usually composed of an inner and outer branch joined near the base. The inner branch (endopod) often has a walking or sensory function while the outer branch (exopod) may be used for cleaning or another purpose. The mouthpart limbs (the mandibles and maxillipeds) also have this biramous structure. Similarly, adult prawns and crabs walk on the inner branch (the endopod) while the outer branch (the exopod) is reduced to a small cleaning rod or has disappeared altogether. The swimming limbs on the abdomen have very similar endopods and exopods. The uropods are the last pair of limbs on the abdomen and, together with the last segment – the telson, make up the tail-fan of the prawn or lobster. The larval development of a spade-like telson without uropods, to an adult tail fan with uropods is another useful trait for recognising larval prawns and crabs. Very basic ('primitive') crustaceans have a pair of biramous limbs associated with every segment of their bodies, from the first antenna to the uropods. Reduction from this basic form, to just a few limbs on a few segments, is one of the most fascinating aspects to the Crustacea, and one of the most useful traits for identification.

Large gelatinous zooplankton are also obvious. They comprise three groups: the jellyfish, salps and comb jellies (ctenophores). Many jellyfish

(medusae) are quite tiny (<1 mm diameter), but distinctively look like miniature versions of adults. Ctenophores are walnut- or pea-shaped balls, with eight longitudinal bands of cilia (the ctene plates, Figure 8.3b). Salps look like little gelatinous barrels, from 2 to 20 mm long (Figure 8.3a, b), while the related larvaceans or appendicularians are simply opaque blobs with a fibrous tail barely attached (Figure 8.2b). Most fish eggs are perfectly round, 0.5–1.5 mm diameter, with a clear transparent egg shell and perhaps a droplet of oil. Fish larvae should catch your attention with a large distinctive fish eye, and then you'll notice the gills and mouth (Figure 8.3). The only things with similar looking eyeballs are the baby squid and octopus (Figure 8.3a). Superficially similar to larval fish, the long and slender arrow worms – the tigers of the plankton – sometimes have large chitinous spines or jaws curving out (Figures 8.2, 8.3).

Finally, there is everything else – usually less than 1 mm and of all shapes – the larval molluscs, beach worms, starfish and sea urchins and many others. This chapter guides you to identify the distinctive shapes. For the zoologically minded, a table of taxonomic classification is provided for all the major zooplankton (Table 8.1). Refer to the recommended reading for further identification to down to genus and species – and sometimes sex.

Table 8.1. Zooplankton summary. This summary includes only dominant marine zooplankton (and excluding freshwater zooplankton). Meroplankton spend only part of the lifecycle in the plankton as larvae or medusae, while holoplankton spend their entire life in the plankton.

PHYLUM, Sub-Phylum Class/subclass Order	Meroplankton e.g. larvae only	Holoplankton
CHORDATA, Urochordata: Ascidiacea (sea squirts) Thalacea, Doliolida (salps) Larvacea (larvaceans)	 larvae – –	 totally, *Thalia, Doliolum* totally, *Fritillaria, Oikopleura*
CHAETOGNATHA: (arrow worms)		totally, *Sagitta*
ECHINODERMATA: Asteroidea (starfish) Ophiuroidea (brittle stars) Echninoidea (sea urchins) Crinoidea (sea lilies) Holothuroidea (sea cucumbers)	(pluteus larva) bipinnaria→brachiolaria pluteus larvae pluteus larvae larvae larvae	– – – – – –

MOLLUSCA:	trochophore→veliger larvae	
Gastropoda (snails and slugs)	larvae	
Prosobranchia	larvae	violet shell, *Janthina*
heteropods		heteropods, *Firoloida, Atlanta*
Opisthobranchia	larvae	
(nudibranchs and sea slugs)		e.g. *Glaucus*
(shelled pteropods or sea butterflies)		totally, e.g. *Creseis*, *Limacina*
(naked pteropods)		totally, e.g. *Clione, Desmopteris*
Bivalvia:	larvae	
Cephalopoda:	larvae	
ARTHROPODA, Crustacea:	(nauplius larva)	
Malacostraca:		
Decapoda (shrimp, crabs)	zoea→mysis→megalopa	–
Stomatopoda	larval stages	–
Isopoda, Amphipoda	larvae or epibenthic adults	hyperid amphipods
Euphausiacea (krill)		totally
Mysidacea (mysid shrimp)	epibenthic adults	–
Maxillopoda	–	
Ostracoda		few, mostly benthic
Copepoda	–	most calanoids, cyclopoids
Cirripedia (barnacles)	Cypris larva	only shed exuvia of adults
Phyllopoda, Branchiopoda	–	
Cladocera (clam shrimp)	– larvae	mostly, *Podon*, *Evadne*, *Penilia*,
Anostraca (brine shrimp)	– larvae, epibenthic adult	*Artemia* in saline ponds
ANNELIDA: Polychaeta (marine worms)	(trochophore→veliger larva)	some specialists, e.g. *Tomopteris*
BRYOZOA:	Cyphonautes larva	
CTENOPHORA: (comb jellies)		totally, *Pleurobrachia*
CNIDARIA:		
Hydrozoa, (including siphonophores)	tiny medusa	*Physalia, Velella*
Scyphozoa (true jellyfish)	Medusa	
Cubomedusa (box jelly)	Medusa	
Anthozoa (sea anemone, coral)	Planula larva	

8.2 COPEPODS AND OTHER SMALL AND ABUNDANT ANIMALS

Copepods account for most of the macroscopic zooplankton in the world's estuaries and oceans (over 9000 species). Copepods are the archetypal zooplankter, growing from an egg, through six larval (nauplius) stages and to a further six juvenile (copepodite) stages before finally becoming a sexually reproducing adult (see Chapter 2). The nauplius larva is common to all Crustacea; it is around 0.5 mm in length sometimes with a single compound eye (Figure 8.4, A1–A6). Nauplii have only two or three pairs of limbs – typically the antennae and the feeding limbs with long setae extending out.

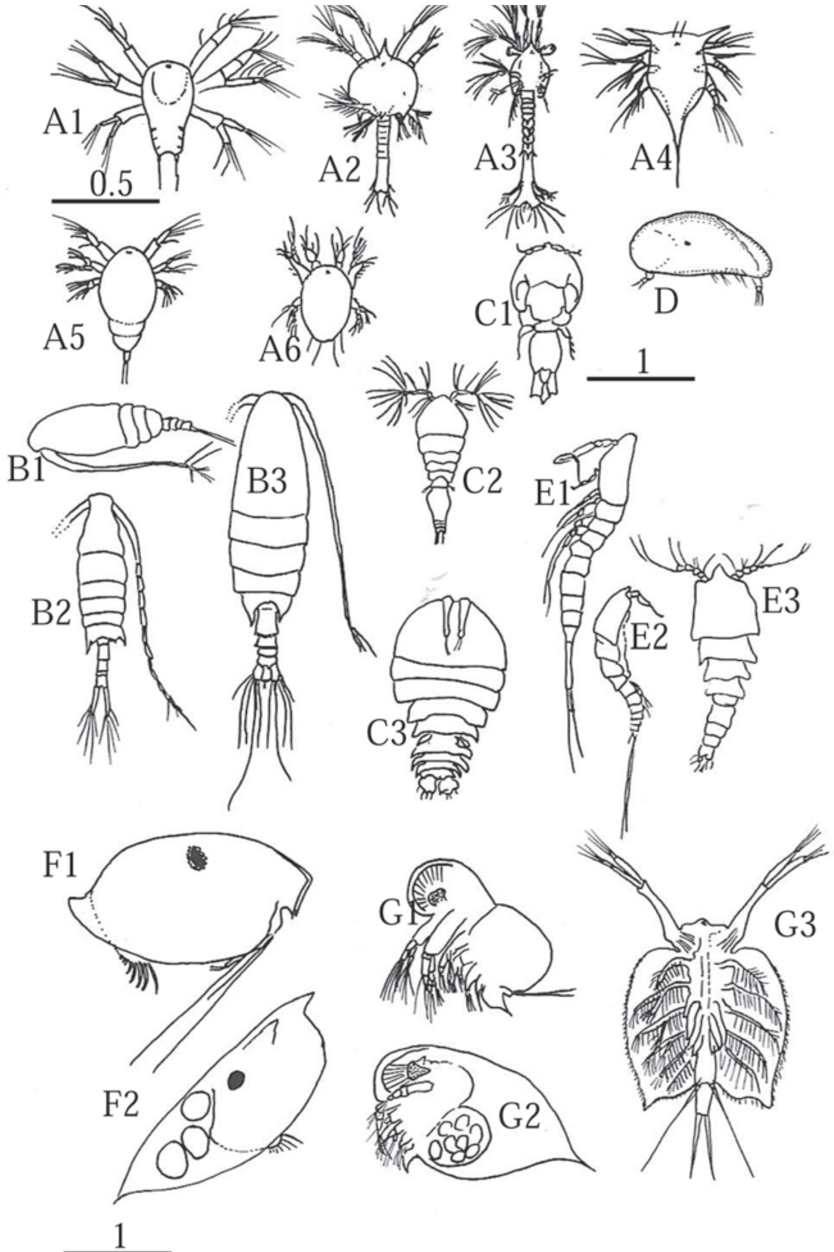


Figure 8.4 Smaller crustacean zooplankton line drawings showing (A1–A6) various nauplii, (B1–B3) calanoid copepods, (C1–C3) cyclopoid copepods, (D) barnacle cyprid larva, (E1–E3) harpacticoid copepods, (F1–F2) ostracods, (G1–G3) cladocerans *Podon, Evadne, Penilia* (Sources: Dakin and Colefax 1940; Wickstead 1965).

Juvenile and adult copepods are small – being 1 to 8 mm in length and with no carapace and a compound, sessile eye. There are no limbs on the abdomen, which is a distinctively thinner 'tail' compared to the thorax. The three pairs of thoracic limbs are well developed for swimming and feeding using comb-like rows of setae, and sinking is controlled by their fat content and extension of the antennae.

Copepods are classified into three major groups (or orders, with three other minor orders) – calanoid, cyclopoid and harpacticoid copepods (Box 8.1).

Calanoids are usually larger and have long first antennae that almost reach the length of the animal and a thinner abdomen (for example, *Acartia,*

BOX 8.1 THREE KEY STEPS TO IDENTIFYING COPEPODS

1) Is it a calanoid, cyclopoid, harpaticoid or something else? Calanoids have long antennae and are larger, while cyclopoids tend to be smaller with very short antennae.
   a) Does it have a movable articulation between the 5th and 6th thoracic segments? Usually with long, strong antennae, nearly as long as body. = **Calanoid** E.g. *Acartia, Paracalanus, Undinula*
   b) Does it have a moveable articulation behind 4th thoracic segment and the metasome is much wider than the urosome? Usually smaller than calanoids with short antennae. = **Cyclopoid** E.g. *Oithona, Oncoea, Corycella*
   c) Does it have a movable articulation behind the 4th thoracic segment, a slightly wider metasome than urosome and both are more or less cylindrical. Usually with a long furcula or setae from the rear, almost as long as itself. = **Harpacticoid** E.g. *Euterpina, Microsetella, Macrosetella*.
   d) None of the above (very rare and parasitic on fish: *Thaumaleus, Monstrilloida, Caligus*).
2) Is it from estuarine or oceanic waters?

   Estuarine samples often contain smaller individuals and are less species rich. E.g. *Oithona, Euterpina, Paracalanus, Acartia, Gippslandia, Gladioferens* (especially at night)
3) To identify a copepod to genus or species when, based on size, shape, general appearance and habitat information, a number of possibilities exist then it is necessary to look at the shape of the 5th legs. To do this, dissect the copepod under the compound or dissecting microscope using a pair of tungsten needles (Box 4.8).

Calanus, Temora and *Gladioferens*, Figure 8.4B). They scatter their eggs into the water, or retain them in a sac until they hatch (Box 8.2). The first stage in identifying them is the number of segments behind the head (three, four or five, Figure 8.4B).

Cyclopoid copepods are often smaller, with distinctively shorter antennae. Females often retain eggs in an ovisac. Cyclopoid copepods include some carnivorous species (such as *Oncaea, Oithona* and *Sapphirina*, Figure 8.4C). Sometimes looking over the side of a boat offshore on a still day, the red and purple iridescent glint off the flattened body form of *Sapphirina* may be seen.

Harpacticoid copepods are smaller still, elongate and with no difference in width between the thorax and abdomen. They have short antennae, egg sacs and are typically benthic – although they may be found in the plankton at night or on drift algae (*Macrosetella, Microsetella*, Figure 8.4E). Some harpacticoids have distinctive very long tail setae – almost as long as the animal.

A related group of small crustaceans are the ostracods (>8000 species) and cladocerans (400 species) – sometimes known as seed shrimps or clam shrimps – which have their vastly reduced bodies and limbs contained within a bivalved carapace. Of the two, ostracods are smaller and often benthic, with the head and eye completely contained within the carapace

BOX 8.2 THE ECOLOGY AND AQUACULTURE OF A DOMINANT ESTUARINE COPEPOD

Gladioferens is a genus of calanoid copepods containing around five species, found abundantly in the estuaries of Australia and New Zealand over a wide range of salinities. It is described as a pioneer herbivore, exploiting the phytoplankton blooms after rainfall (Bayly 1965; Rippingale and Payne 2001). Their abundance is in part regulated by other copepods including the omnivorous predators *Sulcanus* (a cyclopoid) and *Acartiura* (a calanoid). Calanoids seemingly glide through the water, typically upside down, propelled by rapid beating of their second antennae. Jerky swimming may also occur when they rapidly swim with the five pairs of swimming legs. Adult male *Gladioferens imparipes* have a bent left first antennae (that is, they are asymmetric), which it uses to grasp the female and attach a sperm packet near her genital opening. The female releases the fertilised eggs into a sac until the free swimming nauplii hatch. They may complete all six naupliar moults and all six copepodite moults to become a mature adult in 10–12 days at 25°C (Payne and Rippingale 2001). By thriving in estuaries, *G. imparipes* has many natural attributes for aquaculture and as food for larval fish.

(for example, *Pyrocypris* and *Euconchaoecia*, Figure 8.4F). They swim by twirling a powerful pair of antennae that they can retract safely within the halves of the carapace. The Cladocera (Branchiopoda) are best known by the freshwater *Daphnia* or water fleas, which have a head and antennae that are frequently proud of the carapace. The marine equivalent is *Penilia* (Figure 8.4G3), which when dead in the sorting dish, lay on their backs and the two halves of the carapace relax wide open (like the wings of a small butterfly), exposing the limbs. Two other related species, *Evadne* and *Podon*, seem to be 'all eyes and a few limbs' showing remarkable simplification from the basic crustacean form (Figure 8.4G1–G2).

A related group are the larval cirripedes (barnacles), which may be found as dark, dense cyprid larvae ready to settle (Figure 8.4D). Sometimes the large translucent exoskeletons (exuvia) of adult barnacles occur, when they moult *en masse* during warm summer months.

8.3 SHRIMP-LIKE CRUSTACEAN ZOOPLANKTON: LARGER EYES AND LIMBS

The shrimp-like or elongate zooplankton include the larvae of commercial Crustacea, which are familiar to us as prawns (the commercial penaeids), shrimps (everything else similar), lobsters, hermit crabs and crabs (the decapods). The various species and larval stages sometimes have specialised names, but all crustacean larvae begin as a nauplius (Figure 8.4A). To identify the major groups, two key traits to look for are the presence or absence of a carapace and the presence or absence of stalked eyes. The first task with this group is to be able to recognise the small adult shrimps – the krill (euphausids) and the mysids.

Adult krill are recognised initially by their size and abundance, and are typically found in night-time tows. They may have bioluminescent dots along each segment of their abdomen, stalked eyes, a loosely fitting carapace around the abdomen and their long and setose feeding limbs (Figure 8.5A). The larval stages with no swimming limbs are more difficult to identify, and the juveniles may be recognised after eliminating other candidates (below).

Mysids also have stalked eyes, but are nearly translucent (when alive) and more slender, with a looser fitting carapace than the krill (Figure 8.5B). Their remarkable translucence allows one to admire the tubular heart, the gut peristalsis and the many beating limbs (including eight thoracic pairs). Nearly all mysids have a pair of balance organs (statocysts) in the tail fan limbs (the uropods), which appear initially like a pair of translucent

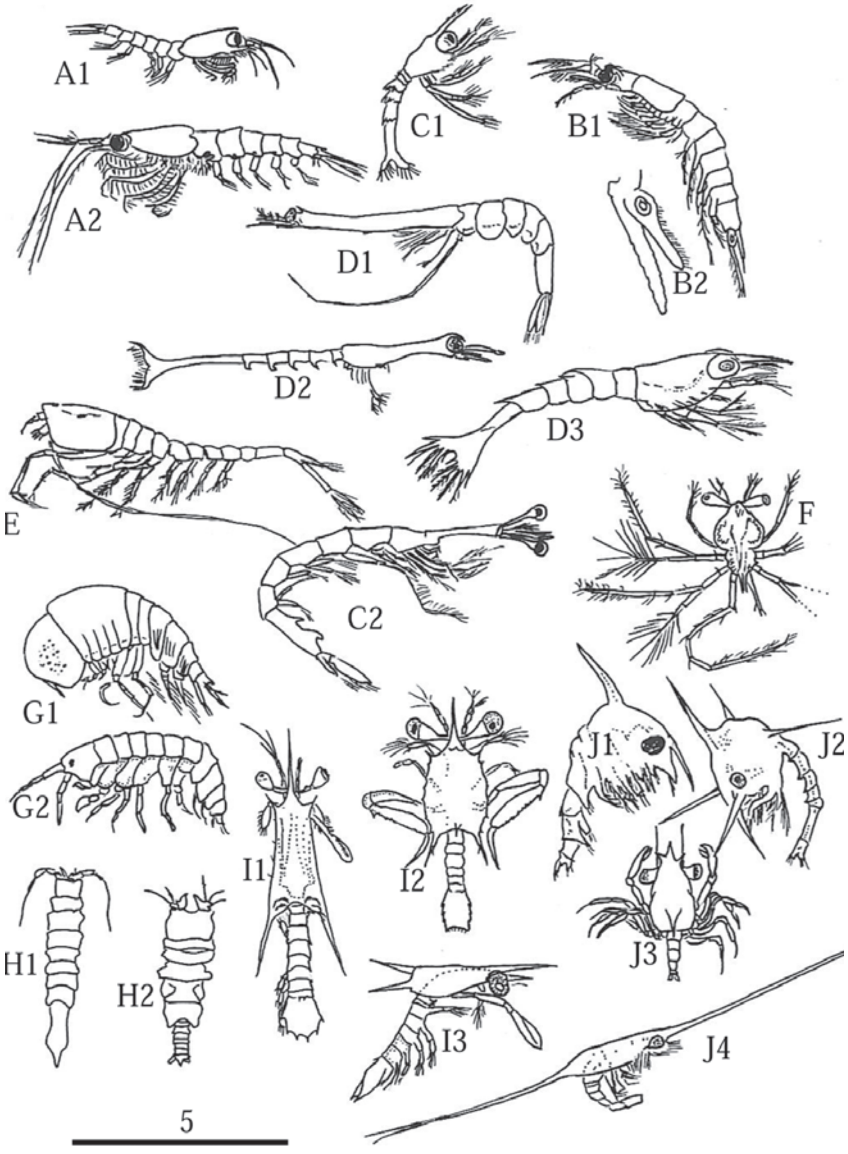


Figure 8.5 Larger crustacean zooplankton line drawings showing (A1–A2) euphausids and various life stages, (B1–B2) mysids including detail of statolith within uropod, (C1–C2) larval penaeids and sergestid shrimps, (D1–D3) anomuran zoea, (E) cumacean, (F) lobster larva, (G1–G2) amphipods, (H1–H2) isopods, (I1–I3) stomatopod zoea, (J1–J4) crab zoea and megalopa (Sources: Dakin and Colefax 1940; Wickstead 1965).

bulls-eyes from a dartboard. Statocysts (unlike the fishes' otoliths) are usually composed of calcium fluorite, with the consistency of toothpaste. Mysids (700 species) are particularly abundant in riverine estuaries, and are the prey of juvenile fish and prawns.

The third group of elongate crustaceans are the many decapod shrimps and their larvae – which also have a carapace and stalked eyes. However, remember that these are larvae, so these can often be identified by the possible lack of swimming limbs (Figure 8.5C, D), or the lack of uropods (just a spade-like telson). This group contains a wide variety of shapes and species:

- Prawns and sergestid shrimps fertilise their eggs externally, which hatch into a nauplius (Figure 8.4A) and thence moult into a zoea or mysis. A distinctive member of this group is the holoplanktonic *Lucifer*, a sergestid shrimp with a stalked head and eyes (so called ghost shrimp, Figure 8.5C2).
- The remaining decapods retain their eggs on the swimming limbs, and the nauplius stage is completed in the egg, hatching into a zoea. There is a wide variety of larval carid shrimps (including Alpheidae, Pandalidae, Hippolytidae and Palaemonidae) that, like many groups in this section, may have distinctive features, but are only useful for recognising genera or species rather than the group as a whole (Figure 8.5D).
- Thalassinids are an under-appreciated group of prawn-like crustaceans, often used as bait and sometimes known as yabbies or mudshrimps. Their larvae also appear as elongate zooplankton (examples are *Jaxea* and *Callianassa*, Figure 8.5D1–D3).

The remaining decapods are those that as adults have a heavy exoskeleton, such as lobsters (Palinuridae, Scylaridae), hermit crabs (Anomura) and crabs (Brachyura). Lobster larvae are outstanding, hatching into large and distinctive zoeae, known as a phyllosoma, which range in size from a few mm across up to 20 mm (Figure 8.5F). Crab zoeae have relatively large globular heads and thoracic bodies, often with large and distinctive dorsal and lateral spines, and quite small limbless abdomens (Figure 8.5J). Crab zoea then moult into a megalopa larva, taking on the appearance of a small crab (Figure 8.5J3).

As a group, crustaceans could be viewed as rather benign. However, adult stomatopods are the jaguars of the crustacean world – often called 'prawn killers' or 'shell smashers'. Adults are relatively intelligent and beautiful, and sometimes quite colourful. Taxonomically they are quite

separate from the above decapods. Stomatopod zoea have a large flared loose carapace (like a translucent cloak or wing) with spines at the corners, and their distinctive spearing limb of the second maxilliped is apparent even in the early larvae (Figure 8.5I).

Only a few crustaceans are found on land and the most successful are the amphipods (beach hoppers) and isopods (pill bugs), which are familiar to us from damp areas in the garden. In the sea, members of these groups are usually benthic, and the females retain their eggs and larvae in a marsupium between their legs, later releasing miniature adults. At night they may swim up into the plankton – as may another related group: the tanaids. These groups have no carapace and a sessile compound eye, and are usually greater than 3 mm in length. The amphipods tend to be compressed laterally (Figure 8.5G) while the isopods are dorso-ventrally flattened (Figure 8.5H). The hyperid amphipods are holoplanktonic, and are characterised by very large eyes (Figure 8.5G1). At night the normally benthic living cumaceans can swarm into the water column to mate and moult. They look superficially like a large calanoid copepod, but with a bulbous head and thorax, and a slender abdomen (Figure 8.5E).

8.4 OTHER LARGE ZOOPLANKTON

There are only about 100 species of ctenophores and nearly all are holoplanktonic (there are a few benthic species). They are major predators of copepods and larval fish, using sticky cells on their pair of tentacles, or lobes around the mouth, to catch their prey. Typical ctenophores are globular – ranging in size from a pea to a golf-ball (Figure 8.6A). They have eight longitudinal rows of cilia (ctenes, or fine hairs), which can be iridescent, giving the illusion of a spinning top. Unlike the true jellyfish, they have bilateral symmetry on top of their radial symmetry, and have sticky – not stinging – cells (known as colloblasts). Like jellyfish, ctenophores have only two basic tissues, inner and outer, separated by a large layer of jelly (mesoglea).

Local estuarine ctenophores are either tentaculate, with a pair of one metre long tentacles (Figure 8.6A, *Pleurobrachia* and *Hormiphora*), or softer-bodied lobate forms without tentacles, but with two large oral lobes (Figure 8.6A2, *Beroe, Bolinopsis* and *Leucothea*). Tentacles of ctenophores may be retracted into sheaths within the body, especially after being caught in a plankton net. Ctenophores release eggs that hatch into (cydippid) larvae, which are less than 0.2 mm long and similar to the adult. Lobate ctenophores are the largest of ctenophores (90 mm bell height) and exceedingly fragile,

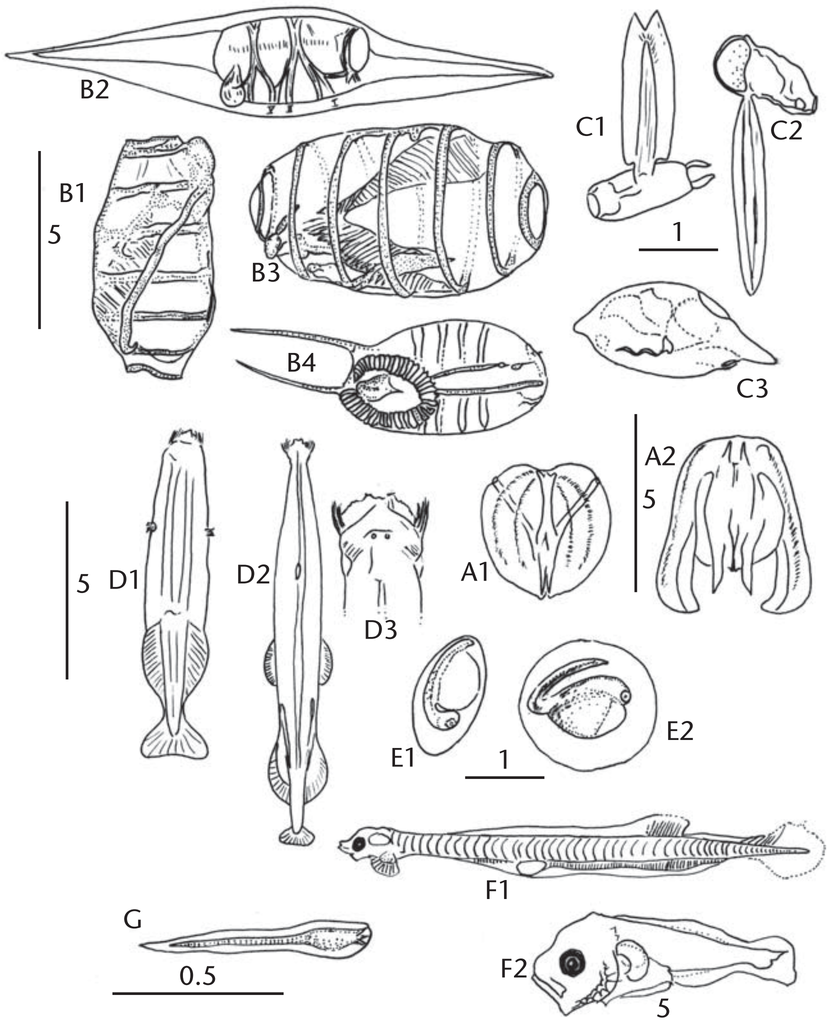


Figure 8.6 Other, larger zooplankton showing (A1) tentaculate and (A2) lobate ctenophore, (B1, B3) doliolids, (B2, B4) salps, (C1–C3) larvaceans, (C3) a sketch of a larvacean inside its house, (D1–D2) chaetognaths with (D3) detail of head, (E1, E2) fish eggs, (F1, F2) larval fish, (G) tadpole larva of a sea squirt or ascidian (Sources: Dakin and Colefax 1940; Wickstead 1965).

sometimes resulting in a puzzling plankton sample of clear amorphous jelly (Box 8.3). They do not preserve well.

Salps, doliolids and the larvaceans (or appendicularians) are the third group of gelatinous zooplankton. They are the specialised pelagic relatives of benthic sea squirts, and indeed ourselves (because these animals possess a notochord – the precursor to a 'backbone', at least during larval development, they are all within the Phylum Chordata). Salps and doliolids are similar, but the former have discontinuous muscle bands around their gelatinous barrel shaped body, while the latter have continuous muscle bands around the 1–2 cm long animal, containing the opaque gut and gonad (Figure 8.6B). At one end of the animal is an inhalant siphon leading to a filtering basket for removing bacteria and very small phytoplankton, with an exhalent siphon at the other end. They have no limbs, tentacles or eyes. Following the phytoplankton bloom, salps tend to bloom during the early spring months by asexual budding. Delicate chains of these animals may be seen in situ – composed of two to dozens of individuals (examples are *Salpa, Pegea* and *Doliolum*). The iridescent cyclopoid copepod *Sapphirina* is often found inside salps (Dakin and Colefax 1940).

Related to the salps are colonies of free floating sea squirts (*Pyrosoma*). They appear as cigar-sized cones or up to 3 m long tubes of 'orange eggs' in shallow waters from southern NSW and Tasmania (but usually occur in very deep water). They are bioluminescent at night.

Larvaceans are even more specialised sea squirts, with only around 60 species. They consist of a tiny spongy ball containing the head, mouth, gut and gonad, which seems barely attached to a very flat fibrous looking tail (1–2 mm in length, *Oikopleura, Fritillaria*, Figure 8.6C). In an undisturbed state, the larvacean constructs a delicate gelatinous house around itself,

BOX 8.3 CTENOPHORE BLOOMS

Ctenophores may sometimes bloom (up to one per litre) and may fill a plankton net making it difficult to retrieve into the boat. Ctenophores are voracious predators, eating over 10 times their body weight in crustacean zooplankton per day, despite their body composed of 96% water. The feeding rate seems to be related to tidal turbulence, bringing zooplankton into contact with the sticky cells on the tentacles or lobes. Once *Pleurobrachia* senses it has 'fly-papered' a copepod onto a tentacle, it spins its body to rapidly wrap its tentacles around the body, somehow wiping the copepod across the single body opening to the central gut. Their abundance varies seasonally and is not necessarily indicative of any environmental concern.

which support very fine primary and secondary filters built into its wall (Figure 8.6C3). The tail generates a filter-feeding current, but eventually the filters clog and the tail helps to inflate a new house from under its mouth. The discarded house may sink to the sea floor and – because six or more houses may be made per day – they are regarded as important components of the global carbon transport, from the atmosphere to the deep ocean. The growth rates of larvaceans are phenomenal, and have been described as the fastest growing animals on the planet. Their central role in the microbial loop and the global carbon flow (Box 8.4) is only surmised as we know very little about this important group.

Chaetognaths, or arrow worms, are holoplanktonic worm-like animals that are placed in their own phylum (Chaetognatha, about 100 species). They are 1–2 cm long, have fins and may initially appear like larval fish without eyes (for example, *Sagitta*, Figure 8.6D). They are predatory, with a row of bristles or spines either side of the mouth, and may sometimes be found grasping another animal. Some oceanographers use them as indicators of a particular water mass.

Fish eggs are usually perfectly spherical, each containing a ball of yolk or embryo delicately suspended inside (Figure 8.6E). An exception is the elliptical anchovy egg. The eggs hatch into larvae with large and distinctive eyes and only fin folds (Figure 8.6F, Section 8.8). The larvae of

BOX 8.4 SALPS, LARVACEANS AND CLIMATE CHANGE

Salps and the appendicularians have been described as the fastest growing metazoans (multi-cellular animals) on the planet (Hopcroft and Roff 1995). They consume tiny phytoplankton and bacteria that are many orders of magnitude smaller than themselves (a much greater size difference than the copepod diet), and produce dense fecal pellets that rapidly sink. Therefore salps have the potential to alter regional food-webs and even global fluxes of carbon via their fecal pellets (Madin *et al.* 2006; Andersen 1998). The most common salp of south-east Australia, *Thalia democratica*, can reproduce both sexually and asexually. An individual may produce a chain of individual clones, resulting in the population doubling or more per day (Heron 1972). Salps compete with other zooplankton such as copepods and krill. In the Southern Ocean, for example, a decrease in krill populations over the last 50 years has been accompanied by an increase in salp populations (Atkinson *et al.* 2004). In sub-tropical waters, the relative abundance of salps in the zooplankton community could alter the balance between those predator species that avoid salps and those fish for which salps are an important component of their diet.

sea-squirts are very small, delicate little fish-like creatures without eyes (Figure 8.6G).

8.5 OTHER ZOOPLANKTON: WORMS AND SNAILS

Holoplanktonic snails include the heteropods and pteropods (literally 'winged foot', because the foot is divided into two flaps for swimming, Figure 8.7D4). Pteropods may be shelled (thecosomate) or naked (gymnosomate) and are related to the often beautiful sea slugs or nudibranchs (Figure 8.7G). Shelled pteropods appear as coiled shells or simple cones, or resemble seeds when the snail has completely withdrawn into its shell (Figure 8.7D). Naked pteropods may initially appear as an amorphous lump, but closer inspection will reveal the foot, a proboscis and palps or tentacles (Figure 8.7E). Pteropods are all predatory, capturing prey and eating it with a rasp-like tongue (radula).

Glaucus (Figure 8.7G) is a planktonic nudibranch, and may be washed ashore with its prey, which includes the harmless *Velella* (a colonial pelagic hydrozoan related to jellyfish) or the related, but far more potent, blue-bottle (*Physalia*). Remarkably, the stinging cells of *Physalia* seem to be grazed undischarged, which *Glaucus* incorporates into its lateral extensions for its own protection.

Heteropods are ecologically similar to pteropods, but are highly modified snails (prosobranch gastropods, Figure 8.7C, *Atlanta*). They are laterally compressed, with a small shell beneath the foot modified into a single ventral fin (i.e. they swim upside down). The female *Firoloida* possess a permanent egg filament protruding from behind (Figure 8.7I). It is nearly translucent except for the gut and eyes, and feeds on small crustaceans and gelatinous zooplankton. It is typical of tropical, offshore zooplankton. The purple snail *Janthina* is a large and holoplanktonic gastropod snail that builds a raft of bubbles for a float and can be washed up on the beach during summer (Figure 8.7H).

Frequently there may be many tiny gastropods and bivalves in a zooplankton collection, which have metamorphosed from larvae to juveniles and are ready to settle onto the bottom (Figure 8.7D). When alive, the small bivalves may be observed each extending out their slender molluscan foot between the shells and flipping themselves around. When dead, they may often be distinguished by the presence of concentric growth lines (Figure 8.7F). Other planktonic molluscs in estuarine samples are rare, but coastal and oceanic samples are rich with squid and cuttlefish larvae or juveniles.

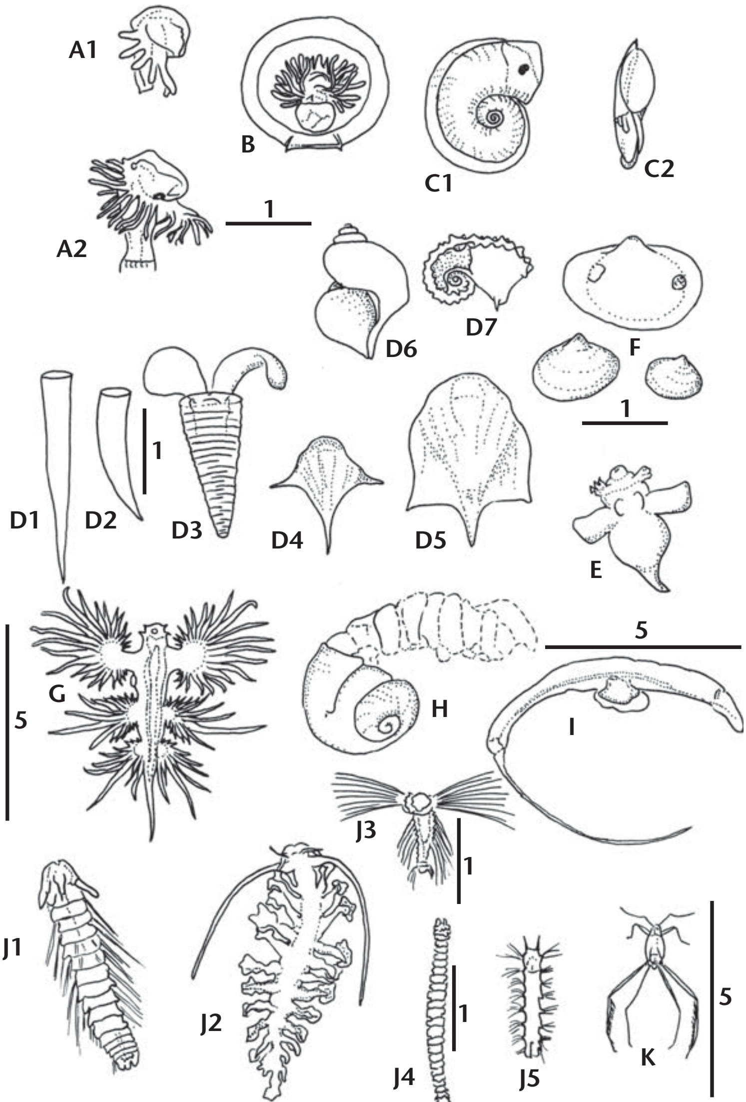


Figure 8.7 Irregular zooplankton showing (A1, A2) actinotroch larva, (B) brachiopod larva, (C1, C2) planktonic snails (heteropods), (D1–D5) other planktonic snails (shelled pteropods), D6 a shelled pteropod and similar appearance to a larval snail, D7 an echinospira larva – the veliger larva of an unusual gastropod snail, (E) a shell-less planktonic snail or naked pteropod, (F) bivalve larvae, (G) *Glaucus*, planktonic nudibranch, (H) the unusual prosobranch snail, *Janthina* with its bubble raft, (I) planktonic snail (heteropod, *Firoloida*), (J1–J4) larval beach worms and pelagic polychaete worms, (K) marine insect, water strider, *Halobates* (Sources: Dakin and Colefax 1940; Wickstead 1965).

The veliger of a beach worm (polychaete – literally 'many chaetae' or small spines) soon begins to grow the many repeated segments characteristic of the true worms (Figure 8.7J). Each segment may have a pair of fleshy limbs (parapodia) with bundles of chaetae extending out. Juvenile polychaete worms may be recognised in plankton samples as they curl up into a ball exposing the many chaetae (like a tiny echidna). Adult polychaetes may also be caught at night when they swim up off the sediments into the plankton, often for breeding. At least one family of holoplanktonic polychaetes are known (*Tomopteris*, Figure 8.7J2), but are relatively rare in our local zooplankton.

Even rarer are the linguilid larvae of the Brachiopoda, and the actinotrocha larva of the Phoronida (Figure 8.7A). Insects in your plankton samples are usually blow-ins, but there is a remarkable water strider that can be found on the surface of the warm oceans, far out at sea (*Halobates*, Figure 8.7K). Sea mites are also known.

8.6 SMALL AND IRREGULAR ZOOPLANKTON (<0.2 MM)

The small, irregular zooplankton remaining in a 200 μm mesh plankton sample are part of a very diverse group, and are of immense importance for the food web. Some comparatively large phytoplankton (less than 0.1 mm) will often be caught up in your sample, but fortunately they are quite distinctive (diatoms, and the dinoflagellate *Ceratium*, Figure 8.8A). Other single-celled animals include various star-shaped radiolarians and beautifully shaped foraminifera (the forams, Figure 8.8B). Radiolarians produce an internal silica test, or shell, with part of the cell extending out through tiny perforations and along spines for feeding (Figure 8.8B, *Acantharia*). Forams produce a calcium carbonate test, which is often altered by temperature or stress. The deposits of these distinctive tests often provide clues to past oceanographic environments, as well as modern day integrators of water quality. While forams are typically benthic, some well known planktonic forms are *Globigerina, Globigerinoides, Neogloboquadrina, Orbulina* and *Turborotalia* (Figure 8.8B2). A third group of protozoans are the cone or vase-shaped tintinnids (Figure 8.8C, *Tintinnopsis, Codonellopsis, Favella* and *Rhabdonella*). When undisturbed, they extend a crown of cilia around the top of the cone, which is able to capture diatoms. The unarmoured (naked) dinoflagellate *Noctiluca* also captures diatoms and other plankton (see Box 1, Chapter 1). It is large (around 1 mm diameter) and entirely carnivorous, and contains no photosynthetic pigments. *Noctiluca* look like translucent, reddish balls (like peaches, with single tentacles,

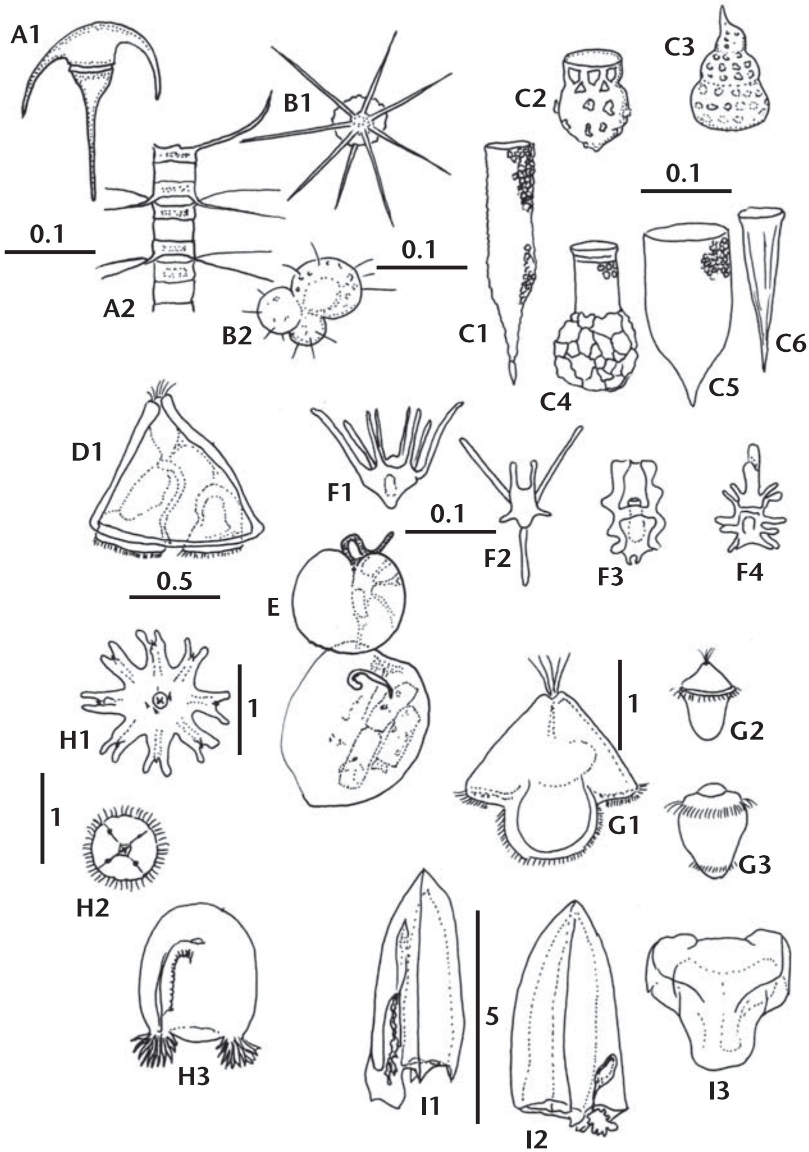


Figure 8.8 Small irregular plankton showing: (A1) large dinoflagellate *Ceratium,* (A2) chain forming diatoms *Chaetoceros,* (B1) radiolarian, (B2) foram or globigerinid, (C1–C5) tintinnids, (D) larval bryozoan (cyphonautes larva), (E) *Noctiluca,* one with diatom prey inside, (F1–F4) larval echinoderms (pluteus larvae), (G1) larval nermetean worm, (G2–G3) trocophore larvae (larval polychaete or mollusc), (H1–H3) jellyfish (cnidarians), (I1–I2) siphonophores, (I3) egg mass (Sources: Dakin and Colefax 1940; Wickstead 1965).

Figure 8.8E). They can bloom in the estuary or coastal ocean in response to their preferred prey – diatoms – which, in turn, have bloomed in response to nutrient upwellings or sewage. *Noctiluca* tend to bloom within a critical temperature range around 20°C and may numerically dominate the zooplankton (Figure 8.8E).

Adult bryozoans form an encrusting sheet of colonial, filter-feeding animals, found on rocks, kelp and any other firm surface. Their larvae (known as cyphonautes larvae) are distinctive little triangular bivalved animals, with a row of cilia along their longer convex side (for example, *Bugula*, Figure 8.8D). Larval bryozoans provide a useful bioassay of heavy metals and other environmental impact assessments.

Echinoderms may be apparent in your plankton sample as distinctive larvae (Figure 8.8F). The larva of a sea star (Asteroidea) is known as a bipinnaria, which is characterised by the growth and folding of the ciliated band to form two loops. This larva settles to become a brachiolaria, with arms and a sucker; it then metamorphoses into the young sea star and frees itself from the remains of its attached larval form. The characteristic larval stage in the brittle stars (Ophiuroidea) and sea urchins (Echinoidea) is the pluteus which has an external apical plate with a tuft of cilia and a single, curved ciliary band. Pluteus larvae are most apparent in 200 μm mesh plankton samples. Larvae of these two groups of echinoderms differ, but, in both, metamorphosis is dramatic and often rapid.

Larval snails and beach worms hatch into a tiny free-swimming, ciliated, trochophore larva around 0.2 mm across (Figure 8.8G). The trochophore stage is followed in the gastropods and bivalves by a veliger larva, with a foot and shell. The veliger then settles to the bottom as a young adult.

There are other phyla not illustrated here, whose larvae occasionally appear in the plankton, including the tornaria larva of the peanut worms (Sipunculida). The remaining zooplankton are the jellyfish and their relatives. There are many small jellyfish among the zooplankton (Figure 8.8H), including the related hydrozoan-siphonophores (Figure 8.8I).

8.7 JELLYFISH AND THEIR RELATIVES

The jellyfish, or medusae, are treated separately here as they are increasingly common, and of great interest to humans because of their sting and as a fishery. Jellyfish belong to Phylum Cnidaria, which is divided into three classes Hydrozoa, Scyphozoa, and Cubozoa (a fourth class of cnidarians contains all the benthic anemones and corals). They are distinguished from all other gelatinous zooplankton, and often from each other, by their stinging

cells (cnidocytes, nematocysts, Ostman 2000). More useful characteristics for field identification include the presence, absence, shape and size of features such as the bell, oral arms, tentacles, stomach, and circulatory canals (Figure 8.9). Variation in these structures leads to organisms as diverse as a lion's mane (*Cyanea*) and a cannonball (*Stomolophus*). Jellyfish range in size from a few millimetres diameter (*Solmundella* or *Obelia*) to over 30 metres long (*Praya*).

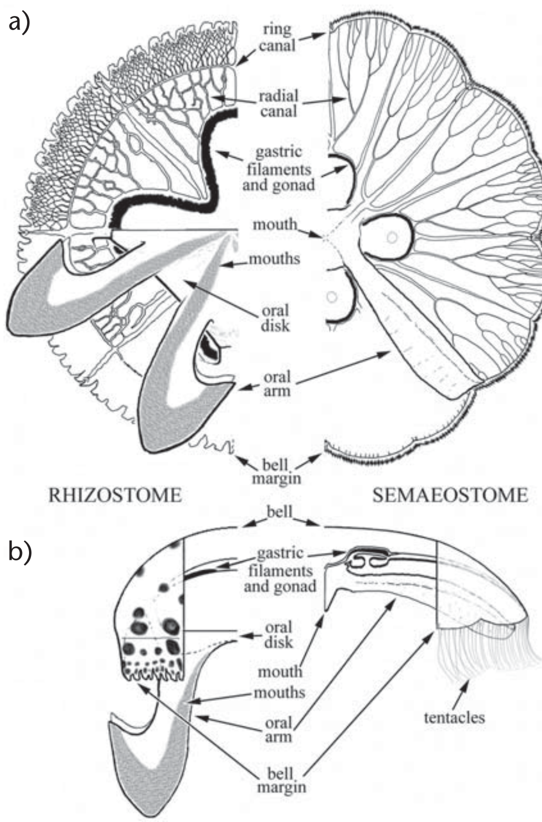


Figure 8.9 Some of the major anatomical features of rhizostome and semaeostome medusae. a) Sub-umbrellar view emphasising features of the oral arms (lower portions) and the bell (cut-away, upper portions). b) Side view of external and, with cut-away, internal features. The shape and size of these and other anatomical features can vary considerably and are used to distinguish among taxa from class level down to species. Rhizostome medusae have eight oral arms partially covered with numerous minute mouths, an oral disk, but no marginal tentacles. In contrast, semaeostome medusae have four oral arms, a single mouth, no oral disk, and generally many tentacles at or near the bell margin.

Worldwide, there are approximately 200 described species of familiar, large jellyfish (scyphozoans) in three orders, although only two orders are relevant here: Rhizostomeae and Semaeostomeae. The bell (usually approximately 10 cm to 1 m diameter) is probably the most obvious structure in most scyphozoans, but it can also be adorned with numerous long tentacles, large oral arms, and other appendages, most notably in rhizostomes and semaeostomes (Figure 8.10).

The rhizostomes are the most taxonomically diverse order of scyphozoans, with approximately eight families, 25 genera, and 90 species described worldwide, mostly in the Indo-West Pacific. The rhizostomes are economically important as fisheries (*Catostylus*; Box 8.5), as introduced species (*Rhopilema*), and as problematic blooms (Box 8.6). The rhizostomes are also the youngest order of jellyfish, raising the question why this group

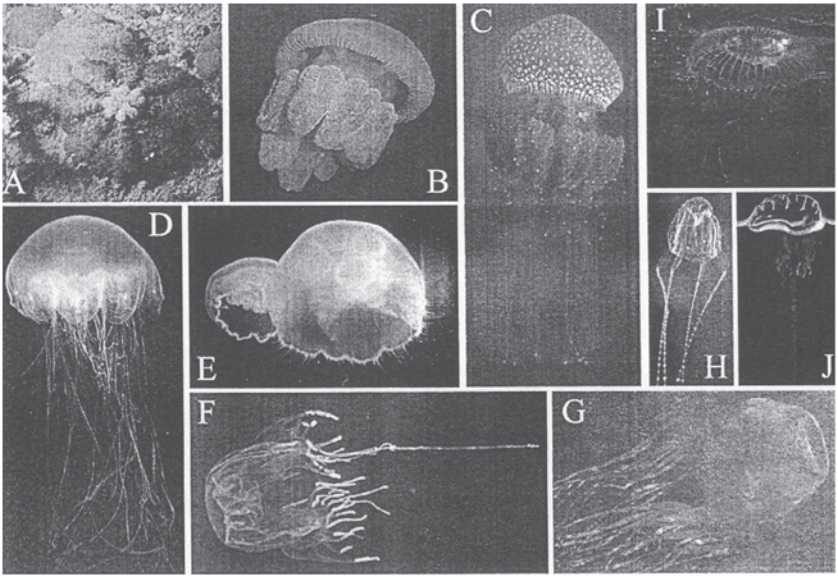


Figure 8.10 Some of the common genera of jellyfishes mentioned in the text that occur in Australian waters. Sizes range from 10 to 30 cm bell diameter.
Class Scyphozoa: Order Rhizostomeae – (A) *Cassiopea,* (B) *Catostylus,* (C) *Phyllorhiza*; Order Semaeostomeae – (D) *Cyanea* (photo G. Edgar, reproduced with permission from Edgar 2000), (E.) *Aurelia*.
Class Cubozoa: Order Cubomedusae – (F) *Chiropsalmus* sp., (G) *Chironex fleckeri* (photo J. Seymour), (H) *Carukia barnesi* (photo J. Seymour),
Class Hydrozoa: Order Leptomedusae – (I) *Aequorea* (photo D. Miller); Order Cystonectae – (J) *Physalia* (photo J. Seymour).

BOX 8.5 JELLYFISH FISHERIES

Dried jellyfish is eaten in many Asian nations and jellyfish have been harvested in China for over 1700 years. Approximately 500 000 tonnes of jellyfish are harvested annually, predominantly in Asia. Increased demand for jellyfish has, however, seen new jellyfish fisheries established in places such as the UK, USA, Namibia and Australia (Kingsford *et al.* 2000). Only rhizostome species, such as *Catostylus mosaicus*, are harvested because they have firm bodies and produce a product that has the desired, slightly crunchy texture. Jellyfish are semi-dried using a combination of alum and salt – the process can take 20–40 days. The dried product is initially prepared by soaking it in cold water to remove the salt. The jellyfish is then shredded into strips, blanched in boiling water and then mixed with sauces and other ingredients, such as chicken, and served cold as a salad. In many countries jellyfish are harvested using large nets or even trawlers, but in Australia fishers may only collect jellyfish using a hand-net. This method is more labour-intensive, but has the benefit of reducing by-catch of undersized jellyfish or other species.

BOX 8.6 JELLYFISH BLOOMS

The profiles of jellyfish blooms – rapid increases to high jellyfish abundance – and their causes have increased worldwide in recent decades (Mills 2001; Purcell *et al.* 2007). For example, blooms of Mediterranean *Pelagia noctiluca* in the early 1980s stimulated international meetings on environmental degradation. A 10-fold increase in the combined biomass of *Chrysaora*, *Cyanea* and *Aequorea* in the Bering Sea from the late-1980s into the 1990s raised concerns about over-fishing, climate change and trophic cascades (Mills 2001). In 2002, large swarms of medusae, which were tentatively identified as *Crambionella orsini*, bloomed in the Gulf of Oman blocking seawater intakes at the Oman Liquefied Natural Gas plant and clogging commercial fishing nets. In these cases, the blooms seem to be attributable to population fluctuations of endemic species. Elsewhere, it is likely that some blooms are due to introduced species, such as *Rhopilema nomadica* in the eastern Mediterranean (Mills 2001). However, all too often, the underlying causes for blooms remain unclear. Integrating data on weather patterns, biological, chemical, and physical oceanography, and jellyfish population dynamics (of both polyps and medusae) should increase understanding of the causes of jellyfish blooms and help mitigate future impacts. In the case of *C. orsini*, there may even be a silver lining because it is an edible jellyfish (Omori and Nakano 2001).

diversified so much so rapidly. Was it their environment or their biology, such as photosymbioses (see Box 8.7), or both that allowed rhizostomes to occupy so many niches?

In contrast to rhizostomes, semaeostomes are the most familiar jellyfish outside the Indo-West Pacific. Worldwide, three families, 18 genera, and 70 species are recognised, including *Pelagia* – one of the first jellyfish to cause international concern over jellyfish blooms (Box 8.6) – and *Aurelia*, the best studied of all jellyfishes. Long thought to be a single cosmopolitan species, *A. aurita* is now known to be a complex of at least 10 cryptic species, which has implications for identifying invasive species, jellyfish blooms, and interpreting decades of research (Dawson 2003, 2004; Dawson *et al.* 2005).

There are relatively few box jellyfishes (Cubozoa): five families, 13 genera and about 30 species described worldwide. They are generally easy to identify as the bell is square in cross-section with tentacles emerging only from the four

BOX 8.7 JELLYFISH SYMBIOSES

Jellyfish have symbiotic relationships with many other organisms. Some species of jellyfish contain photosynthetic dinoflagellates (zooxanthellae) within their tissues, as reef corals do (Figure 8.11A). The degree to which jellyfish derive their nutrition from their photosymbionts varies, with some species being only partially autotrophic (such as *Cassiopea*; Hofmann and Kremer 1981), while others are almost fully autotrophic (such as *Mastigias* in Palau; McCloskey *et al.* 1994). In some cases, the presence of symbiotic zooxanthellae is thought to be responsible for some remarkable behaviours displayed by jellyfish. For example, in the jellyfish lakes of Palau, *Mastigias* migrate along the length of the lake during the day to avoid shadows and maximise exposure to sunlight (Hamner and Hauri 1981). Another species, *Cassiopea* is known commonly as the 'upside-down' jellyfish because, unlike most medusae, it rests upside-down on the bottom to expose the zooxanthellae in its oral arms, to sunlight. A different type of symbiosis involves the association of fish and sometimes invertebrates (such as amphipods, barnacles and crabs) with medusae. Often large numbers of juvenile fish are seen swimming close to jellyfish and small crabs, copepods and other crustaceans can sometimes be found riding on the bell of jellyfish (Pagès 2000; Figure 8.11B). Jellyfish probably provide effective protection against predation for these animals. How these animals avoid being stung by the jellyfish is not known. They may simply avoid contacting the tentacles or, as hypothesised for clownfishes that live in sea anemones, they may have some form of chemical or immune protection.

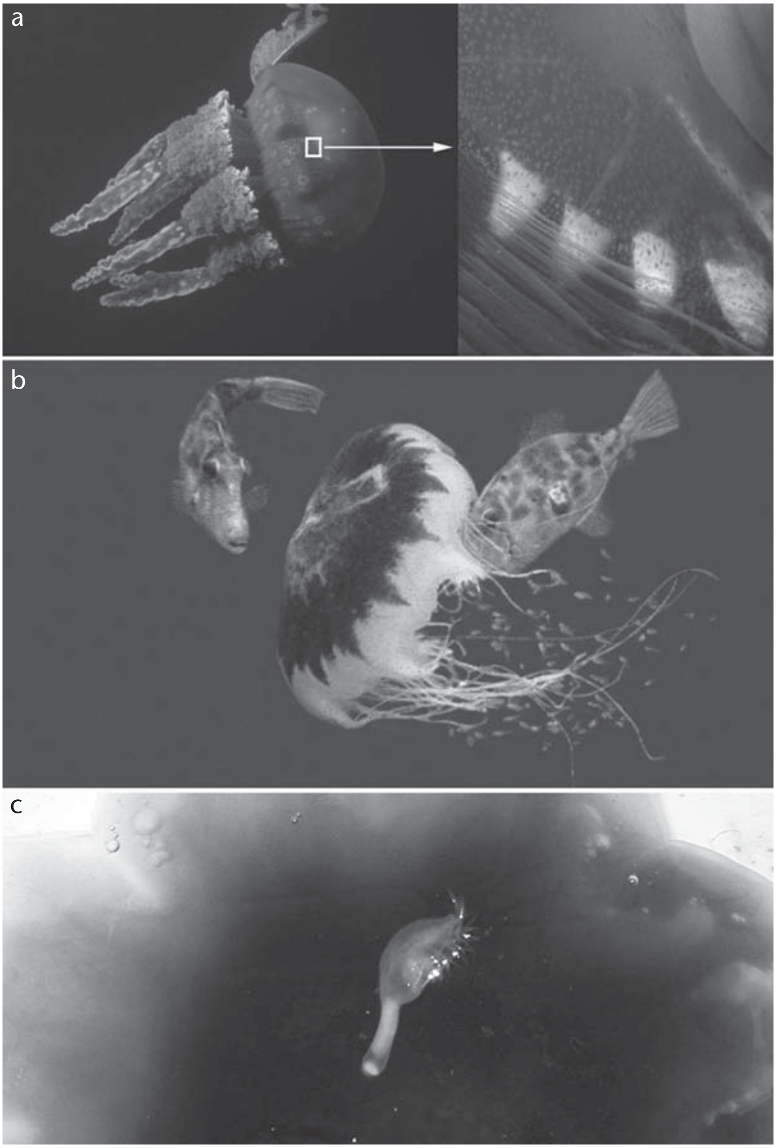


Figure 8.11 a) *Mastigias* from Palau, left, and a close up of zooxanthellae clusters on the underside of the bell, right, showing concentrations of zooxanthellae on muscle bands along the lower edge of the picture and around the gut in the upper right-hand corner. **b)** Small juvenile yellowtail horse mackerel (*Trachurus novaezelandiae*) swimming among the tentacles of the semaeostome jellyfish *Desmonema* in New Zealand, the two large leatherjackets (*Parika scaber*) are preying on the medusa (photo M. Kingsford). **c)** The parasitic gooseneck barnacle *Alepas* on the bell of *Cyanea* caught in the Huon estuary, Tasmania (Tubb 1946).

corners of the bell, and a rhopalium (eye) set in the lower-middle of each of the four sides. Box jellyfish include *Chironex fleckeri* – the most venomous marine animal – and *Chiropsalmus* (Halstead 1988; Nagai *et al.* 2002).

Most of the planktonic cnidarians are hydrozoans. Worldwide there are approximately 45 families, 200 genera, and 700 species of hydromedusae, plus 15 families, 45 genera, and 150 species of siphonophores. Compared with the scyphozoans, many are relatively inconspicuous because of their small size or habitat (under rock ledges or wharves). There are a few obvious exceptions, such as *Aequorea* (order Leptomedusae), which grows to about 15 cm bell diameter and is common in near-shore waters and *Physalia*, the colonial blue-bottle (or Man-o'-war; order Cystonectae), with its distinctive float, or pneumatophore, which is often blown onto the shore. *Physalia* is of particular interest because it causes tens of thousands of stings each year in Australia, South Africa, and the eastern United States (Box 8.8, Box 8.9). Other colonial hydrozoans washed up on beaches are distinctively blue, coin-sized discs with polyps beneath (*Velella*, 'sail-by-the-wind' and *Porpita*). Sometimes clear firm gelatinous cubes or shapes can be found, which are the reproductive stages of siphonophores (Figure 8.8I3).

Most jellyfishes are meroplanktonic (only in the plankton for part of their lifecycle) and have a second life-history stage – the bottom dwelling polyp. One major group (Stauromedusae) occurs only as polyps. Polyps are similar in form to anemones and corals (which comprise the cnidarian class Anthozoa), but are rarely larger than a few millimetres, and are generally benthic. They reproduce asexually to generate other polyps or new medusae

BOX 8.8 THE BLUEBOTTLE, *PHYSALIA*, AND ITS RELATIVES

The bluebottle is often seen in the summer surf and washed up on beaches associated with an on-shore wind. It can inflict a painful sting (see Box 8.9 on treatment). Its habitat is the surface water of the open ocean, where it stings and consumes small fish and zooplankton. It is a holoplanktonic, colonial hydrozoan (one of the classes of cnidaria). The colony is dominated by a highly modified polyp, which forms the float, and other polyps (or zooids) which are specialised as long, stinging polyps, short feeding polyps and thick reproductive polyps. The long stinging polyps have many stinging cells, which can discharge when touched by something the jellyfish does not recognise as itself. A related blue hydrozoan also washed up is the 'sail-by-the-wind' (*Velella*) – a blue disc about the 2–3 cm across with a small triangular sail. *Velella* is harmless to humans, although it also stings and feeds on plankton.

BOX 8.9 HANDLING JELLYFISH: A NOTE ON SAFETY

Most jellyfish stings are not lethal, but a few are. Many more cause rashes, swelling and other symptoms such as nausea, sweating, muscle and joint pain and difficulty breathing (Fenner 1997). Wear rubber gloves when handling jellies and avoid water into which cnidocytes might have been released. Wear a wet-suit (with gloves, booties and hood) if swimming with them. As jellyfish are generally fragile, avoid taking them out of water. Instead capture and move them in bags and buckets.

An effective and practical treatment for pain from bluebottle stings is immersion in warm to hot water (45°C for 20 minutes), which is more effective than the traditional icepack method. Many marine venoms are heat labile and are quickly denatured by moderate heat (Loten *et al.* 2006).

(which usually have separate sexes producing eggs or sperm) depending on environmental conditions. This is one reason why massive blooms of medusae can seem to appear out of nowhere – in reality, they're coming from minute asexually reproducing polyps – which causes problems for coastal management (Box 8.6).

8.8 LARVAL FISH IN ESTUARINE AND COASTAL WATERS

Nearly all fish have an early pelagic larval stage and thus comprise an interesting component of zooplankton samples. Most fish larvae hatch from pelagic or demersal eggs (that is, attached to sand, rocks or seaweed), but there are a few live-bearing species. The larval stage usually lasts 3 to 4 weeks. The presence of fish larvae can indicate important spawning areas, or fish biodiversity, and therefore larval diversity may have greater relevance than mere presence of a transient adult. For example, south-eastern Australia has a large diversity of fish in late summer, because of larval transport from the Great Barrier Reef, yet it is its role as a spawning location that is important for conservation efforts. The source, supply and sinks of larvae are vital components for managing fisheries and the establishment of marine parks (Box 8.10).

Based on their life history, the majority of fish larvae caught in estuaries can be categorised as estuarine or marine opportunists, with low numbers of freshwater or marine straggler species (Potter *et al.* 1990). Estuarine species, which are usually small as adults, spawn, and spend their whole life cycle, in the estuary. In contrast, estuarine opportunist species spawn at sea, usually in coastal waters, with the larvae entering estuaries where the juveniles settle into nursery habitats such as seagrass beds and mangroves. Adults of these

BOX 8.10 LARVAL FISH CONDITION AND DEFORMITIES

The larval stage of fish is considered a bottleneck for fisheries, through starvation of the larvae (insufficient nauplii as food), predation (from jellyfish, ctenophores, krill or fish) and unfavourable currents. Over 99% of the eggs and larvae do not survive, and therefore rapid growth may enhance survival by reducing the duration of the vulnerable larval stages. Consequently fisheries biologists estimate age and larval growth from the width of the daily growth increments of the earstone or otolith (which are analogous to tree rings). Even body width or weight are useful indicators of larval condition, in response to environmental conditions (water temperature or pollution). Fish larvae are very delicate – without scales – so they are susceptible to poor water quality from urban run-off, acidic water or sewage effluent. The incidence of deformities in newly hatched larvae, relative to a control group, is a useful measure of water quality.

species remain in estuaries, only leaving the estuary to spawn or permanently migrate out of the estuary to coastal reefs. Small numbers of larvae of freshwater and marine straggler species can also occur in estuaries depending on the degree of input of marine or fresh water into the system.

Estuarine-spawning species only a short life cycle of 1–2 years and have a number of reproductive strategies to reduce the mortality of eggs and larvae. These strategies include being live bearers, such as the pipefish and seahorses (sygnathids, where larvae develop in a pouch on the males), and the apogonids (mouth brooders) with juveniles hatching at an advanced stage of development. Gobies, blennies, hemiramphids and atherinids have benthic eggs that are attached to seagrasses or other hard substrates such as mollusc shells. Some herring, and other fish with pelagic eggs, spawn in the upper reaches of the estuary to minimise the chance of the eggs being washed out of the estuary. Such strategies mean that larvae hatch at an advance stage of development, which allows them to be retained within estuaries and not be carried out by ebb tides.

The abundance of larvae of estuarine species usually shows a seasonal pattern, with highest abundances in summer and the lowest in winter. The seasonal variation in abundance closely follows the cycle of water temperatures. The abundance of larvae of marine opportunist species entering estuaries also shows a similar, but less-marked, seasonal variation. This is due to the larvae of taxa such as sparids, girellids and scorpaenids entering estuaries during winter.

Species diversity of fish larvae generally decreases with increasing distance upstream, away from the mouth of the estuary. Although samples

from estuarine stations usually have a much lower diversity compared with marine stations, abundances of larvae of estuarine species are usually much higher than for larvae of marine opportunist species entering the estuary on the flood tide (Neira and Potter 1994).

Most estuaries in southern Australia and southern Africa are microtidal, with a narrow entrance channel opening into a large basin or basins. Tidal movements can carry larvae into and out of the estuary. Surveys of larvae in these estuaries report higher abundances of larvae from marine-spawned eggs on flood tides and higher abundances of larvae from estuary-spawned eggs on ebb tide, with higher abundances of larvae at night irrespective of flow direction (for example, Whitfield 1989; Neira and Potter 1992; Trnski 2001). Higher abundances of larvae in surface waters of estuaries at night are due to diel vertical migration of larvae through the water column. Possible reasons for diel vertical migration may be that it reduces predation risk or increases prey densities if larvae occur deeper in the water column during the day and near the surface at night.

Larval fish usually have a very different morphology compared with the pelagic or demersal adults, making them very difficult to identify. Recently, a number of larval fish identification guides have been produced that illustrate the development of larvae from different geographical regions. These identification guides have described larvae, to at least family level, of the majority of species that occur in estuarine and coastal marine waters (for example, Fahay 1983; Moser *et al.* 1984; Ozawa 1986; Okiyama 1988; Olivar and Fortuño 1991; Moser 1996; Neira *et al.* 1998; Leis and Carson Ewart 2000).

The most common method of identification of unknown fish larvae is the series method. This involves identifying the largest available larval or juvenile specimen in the samples, based or adult characteristics such as fin meristics and vertebral number (equivalent to the number of myomeres or muscle blocks – in larvae). The largest specimen is then linked to smaller specimens in the series by using morphological and pigment characteristics. A variety of characters can be used to identify fish larvae including general morphology, such as body shape, gut length and degree of coiling, number of myomeres, pigmentation patterns (melanaophores), the sequence of development of fins and the pattern of head spination (Table 8.2).

The length and stage of development (Box 8.11) are important features in identification. The gas bladder, which is present in many larvae is absent in adults (such as gobies). During the day, the gas bladder may be small in larvae, but strongly inflated and conspicuous in larvae caught at night, which can result in larvae of the same species appearing

Table 8.2. Key identification of features of fish larvae occurring in estuaries (based on information from Leis and Carson Ewart 2000 and Neira *et al*. 1998)

Family	Estuary (E) or Marine (M)	Features
Gobiidae (goby)	E/M	24–34 myomeres; body elongate to moderate; lightly to heavily pigmented; gut moderate and slightly coiled; conspicuous gas bladder. (Figure 8.12K)
Atherinidae (hardyhead)	E	35–47 myomeres; body very elongate; moderately pigmented; gut coiled and compact. (Figure 8.12D)
Hemiramphidae (garfish)	E	51–57 myomeres; body very elongate; moderately to heavily pigmented; gut very long. (Figure 8.12C)
Clupeidae (herring, sprat)	E/M	41–55 myomeres; body very elongate; lightly pigmented; gut very long. (Figure 8.12B)
Engraulidae (anchovy)	E/M	38–47 myomeres; body very elongate; gut very long; lightly pigmented. (Figure 8.12A)
Ambassidae (glass perchlet)	E/M	24–25 myomeres; body depth moderate; lightly pigmented; gut coiled and compact; conspicuous gas bladder; small preopercular spines. (Figure 8.12P)
Sygnathidae (pipefish, seahorse)	E	Elongate body; prominent dermal plates; moderately to heavily pigmented. (Figure 8.12J)
Blenniidae (blenny)	E	Typically 30–40 myomeres; body elongate; lightly to moderately pigmented; gut short and coiled; moderate to large teeth; none to large preopercular spines. (Figure 8.12I)
Gerreidae (silverbiddies)	M	24–25 myomeres; body depth moderate; lightly pigmented; gut moderate coiled and compact; prominent ascending premaxillary process; small preopercular spines. (Figure 8.12O)
Sparidae (bream, porgy, tarwhine)	M	24–25 myomeres; body depth moderate; lightly pigmented; gut moderate, coiled and compact; small to large preopercular spines. (Figure 8.12L)
Girellidae (blackfish)	M	26–27 myomeres; body depth moderate; lightly to moderately pigmented; gut moderate, coiled and compact; small preopercular spines. (Figure 8.12S)
Monacanthidae (leatherjacket)	M	19–20 myomeres; body deep and laterally compressed; moderately to heavily pigmented; gut moderate, coiled and compact; prominent dorsal and pelvic spine with barbs. (Figure 8.12E)
Monodactylidae (moonfish)	M	24 myomeres; body deep and laterally compressed; moderately to heavily pigmented; gut moderate, coiled and compact; large early forming pelvic fins; large preopercular spines. (Figure 8.12R)

Mugilidae (mullet)	M	24–25 myomeres; body depth moderate; heavily pigmented; gut long and coiled; small preopercular spines. (Figure 8.12U)
Platycephalidae (flathead)	M	27 myomeres; body depth moderate; moderately pigmented; gut moderate to long and coiled; large and early forming pectoral fins; extensive head spination. (Figure 8.12H)
Scorpaenidae (scorpionfish)	M	24–28 myomeres; body depth moderate; moderately pigmented; gut moderate to long and coiled; large early forming pectoral fins; extensive head spination. (Figure 8.12G)
Silliganidae (whiting)	M	32–45 myomeres; body elongate; lightly pigmented; gut moderate to long and coiled; very small preopercular spines. (Figure 8.12N)
Terapontidae (trumpeter)	M	25 myomeres; body elongate; lightly pigmented; gut coiled and moderate; small preopercular spines. (Figure 8.12M)
Callionymidae (dragonet)	M	20–22 myomeres; body robust and moderately deep; heavily pigmented; gut coiled and moderate to long; one large preopercular spine. (Figure 8.12T)
Paralichthidae (flounder)	M	33–39 myomeres; body moderately deep and laterally compressed; moderately pigmented; gut coiled and moderate to long; small preopercular spines (Figure 8.12F).

BOX 8.11 DEVELOPMENTAL STAGES OF LARVAL FISH

One of the most commonly used terminologies to describe the development of larval fish is based on that used by Ahlstrom and his co-workers (Moser *et al*. 1984; Neira *et al*. 1998; Leis and Carson Ewart 2000). The larval stage is defined as the development stage between hatching (or birth) and the attainment of full external meristic complements (that is, the number of fin rays and scales) and loss of specialisation for pelagic life. The larval stage is divided into preflexion, flexion and postflexion stages that are related to the development of the caudal fin and the corresponding flexion of the notochord. For example, two contrasting fish larvae show the relative size and stage of development.

	herring	bream
Preflexion	6 mm	3 mm
Flexion	12 mm	6 mm
Postflexion	18 mm	10 mm

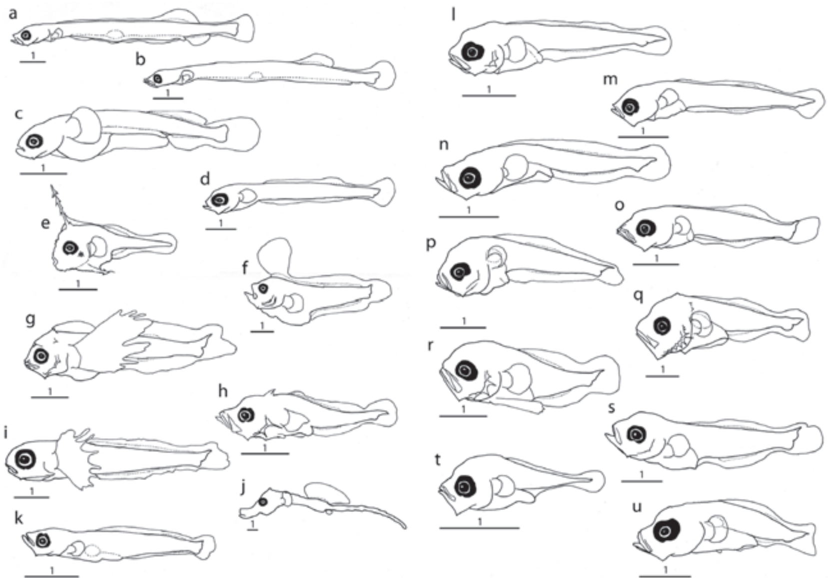


Figure 8.12 The flexion stages of some dominant families of fishes typically occurring in standard estuarine plankton collections: A–Engraulidae (anchovies), B–Clupeidae (herring, sprat), C–Hemiramphidae (garfishes), D–Atherinidae (hardyheads), E–Monacanthidae (leatherjackets), F–Paralichthyidae (flounders), G–Scorpaenidae (scorpionfishes), H–Platycephalidae (flatheads), I–Blenniidae (blennies), J–Sygnathidae (pipefishes, seahorses), K–Gobiidae (gobies), L–Sparidae (bream, tarwhine), M–Terapontidae (trumpeter), N–Sillaginidae (whiting), O–Gerreidae (silverbiddies), P–Chandidae/Ambassidae (glass perchlets), Q–Carangidae (trevallies), R–Monodactylidae (moonfish), S–Girellidae (blackfishes), T–Callionymidae (dragonets), U–Mugilidae (mullets). (Sources: Leis and Carson Ewart 2000; Neira *et al.* 1998).

different depending on when they were caught. The inflation of the swim bladder is related to the diel vertical migration that larvae of many species undertake in estuaries.

Fish eggs are typically between 0.5 mm and 1.5 mm diameter, and are translucent with a clearly defined yolk, or embryo or oil globule(s) (invertebrate eggs are often dark and <0.5 mm diameter). Compared with larvae, there has been very little work undertaken on the identification of fish eggs. The characters that can be used to identify eggs are the egg size and shape, number, position and pigmentation of oil globules, the degree of yolk segmentation, chorion morphology, perivitiline space and embryonic characteristics (for example, Ahlstrom and Moser 1980).

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8.10 FURTHER READING

Many zooplankton and larval fish identification books are available as a CD or even online.

http://www.zooplankton-online.net/index.html

http://www.ices.dk/indexfla.asp

http://www.pac.dfo-mpo.gc.ca/sci/OSAP/projects/plankton/default_e.htm

Some of the classic books noted below are available on the National Marine Fisheries Service, La Jolla website as free pdf http://swfsc.noaa.gov/publications/swcpub/qrypublications.asp

A basic online key for Tasmanian zooplankton may be found at http://www.tafi.org.au/zooplankton/.

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Chapter 9

Models and management

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9.1 INTRODUCTION TO MODELS IN MANAGEMENT

A model is a simplified representation of part of the real world. Models are generally developed to help to understand the major processes taking place within a system, or as tools for prediction. The use of models can help managers make decisions – providing them with an understanding of what the potential outcome of a decision might be and to help isolate the causes of such an effect. Models help the adaptive management process by allowing trial and error of potential solutions before decisions are made. Probably the most common predictive models used by the community are weather forecasts. These forecasts are generated through a variety of complex models that are based on an advanced understanding of the physical system, and using long-term trend data and data available from field measurements (local temperature, air pressure) and measurements from satellites.

Simple conceptual models are similar to cartoons or flow diagrams: outlining the processes that are considered important, without getting caught up in the details. More sophisticated models, such as hydrodynamic box models or

biogeochemical budgets in ecological studies, quantify processes in a simple, often time-averaged manner. Even more sophisticated models, such as hydrodynamic and processes-based ecological models, are built on a collection of quantitative descriptions of the rates of processes. The level of sophistication adopted in a modelling exercise should be dictated by the level of understanding of the processes within a system, the data available for model assessment and the desired outcomes. Often, models of differing sophistication will be employed in the one project, each providing an alternative view.

Models are frequently used by managers involved with decision making about water quality, or who require a simplified understanding of what processes are driving a particular water-quality issue such as an algal bloom. For example, information on the residence times of water in different parts of an estuary indicate where algal blooms are more likely to occur, or where nutrients discharging into a system are more likely to cause a phytoplankton bloom. In this case, the water residence time must be greater than the phytoplankton cell division rates ('doubling time') for a bloom to occur. By simplifying the complexities of real systems, managers determine the most important physical, biological or chemical drivers of a system and can therefore identify where a management intervention may be useful. Managers can also use models to predict what the outcome of a management solution might be. This can save resources and time.

Models can be data hungry and can be expensive to develop or to run. There is a wide range of free or commercial models that can be used, and models can also be custom built. Selecting the right models to address their concerns or questions can be difficult for non-specialist users or managers. To aid model selection, and to ensure that models are appropriate for the purpose, it is essential that consideration is given to a number of issues associated with models and their use.

This chapter discusses aspects that should be considered by model users before developing or applying models. It also provides some examples of models and discusses the application of these models.

9.1.1 Define needs for model

Before starting the expensive process of model development, users of models should define their requirements from a model. It may not be necessary to have the skills to develop, or even to use, models. It is important however, to be able to manage the process of model development and ensure that project needs are achieved. It is possible to influence the model development and to work with modellers to select appropriate models and approaches. It is also important for users to be able to interpret the results

from models and the uncertainty associated with the results and hence the risk of relying on the output.

Effective pre-model planning should include:

- identification and clear articulation of the problem
- a clear understanding of the role that the model will play in solving the problem
- knowledge of the spatial and temporal scales that need to be covered by the model.

Unless the user's needs are defined clearly, it is difficult for the modellers to suggest an appropriate approach. It is important to be aware of the level of uncertainty that may be associated with the results or predictions of the model, and therefore the risk of acting on the results. The more complex the problem, the greater will be the uncertainty associated with the model's output (unless the model is underpinned by wide ranging and detailed data). This uncertainty is generally related to the data requirements of the model and the lack of good quality information about the particular attributes being examined. For example, ecological processes are variable and dynamic, which influences the ability of a model to represent them.

9.1.2 Determine how information will be used

There is no point in having a model if the information from the model will not be used, or will not add value to the work being undertaken. Consideration should be given to how long the model should be useable. If you are aiming to use the model on a regular basis, you may need to be trained in the use and interpretation of the model and to be able to update it as better information comes to hand. Alternatively, you need to ensure access to expertise to enable models to be run, updated and interpreted as required. This can have a significant cost. It is also important to be aware of any issues associated with the licensing of models.

9.1.3 Establish a budget and timeframe

Models can be expensive to develop and to operate. This is generally related to the complexity of the model and the number of complex interactions underpinning them. While modern computers make running complex models easier, the run time of models is still important and costly. It is useful to determine how much you are willing to spend on the development of the model you require. Remember that the confidence that you have in your model output will be influenced by the quality and relevance of data

underpinning the model, and the timeframes in which you are willing to work. Some models can be relatively quick to establish, operate and generate results, while others might be more complex and may require additional data to be collected to inform them. Select models that meet your needs. More time spent planning the project will save time later and reduce costs.

Consider the data and process understanding that are available for the system that will underpin the model. The accuracy of models depends on the quality of the information underpinning it. Quality of data is determined by aspects discussed in Chapter 4 and by the spatial and temporal scales at which the data were collected. Longer periods enable trends to be incorporated into models and for greater certainty in their predictions.

It is important to consider what additional information may be required and what the timeframe and costs of collecting this information will be. This can include considering other sources of data for the model and how these data will be integrated into the model. If the model will be used over long periods of time, how will new information be integrated into the model to ensure that it remains useful? Long-term data collection programs can be designed to support the continued use of model and to reduce the levels of uncertainty associated with the model's predictions.

9.1.4 Calibration, certainty and relevance of models

Model assessment measures the accuracy of the model. Calibration, or the more sophisticated techniques of data assimilation from tide gauge or satellites, can be used to 'determine' the accuracy. Calibration is when the underlying parameters are adjusted to ensure that there is strong correlation between the information measured in the field and the results from model simulations. Good correlations signify that the model results are meaningful, which can increase the certainty that users might have in the model output. When beginning the process of model development, it is important that model calibration is considered when deciding on time and budgets.

Users should have a thorough knowledge of any assumptions made in the production of the model. By undertaking a reality check of model output before acting on that output, managers can reduce the distrust and cynicism about model predictions that often exists in the broader community. In some cases, such as hydrodynamic models, reality checks may be simply assessing the correlation of collected versus predicted data. If the correlation is strong enough, and is based on a sufficiently large data set (many points), then the model can be considered to be sufficiently

accurate. In more complex decision support models with a variety of variables, often based on poor quality data or expert opinion as well as on a range of complex interactions, reality checks can indicate where spurious predictions are made. Models can then be assessed to determine which variables resulted in those outcomes. As well as getting more realistic predictions from models, reality checking can provide other useful information to managers. If outcomes are counter-intuitive, and are shown to be based on poor quality data or information with a high level of uncertainty, then managers are able to focus data collection or knowledge generation on those specific variables and improve the quality of the model. A useful approach is to undertake a risk analysis when developing models. Assessing the risk of acting on information obtained from a model when little is known about an uncertainty is important.

Communication helps stakeholders to be aware of models that are being developed, why a particular model is being developed, how the models will be used and what data will be gathered to underpin the model. If stakeholders are confident in the processes leading to a model's prediction, they will be more likely to act on that prediction.

The next section provides examples of some of the models used to support understanding and management of some aspects of aquatic science. These have been selected to illustrate the range of models and to show how the output of these models can be useful to managers. These sections should be considered in light of the discussion above. Models are complex and often difficult to understand, but can help managers immensely in making decisions. We provide here details of two trophic models (Sections 9.2, 9.3) and a description of a decision-support model (Section 9.4).

9.2 EXAMPLES OF TROPHIC MODELS

Trophic models set out to describe the dynamics of a food web. A number of key terms are described in Table 9.1. The most typical forms of food web models, with applications in terrestrial as well as aquatic ecosystems, are *predator–prey* models. Predator–prey models (or 'Volterra' models) capture the oscillations that can occur in the abundance of predators and prey (such as foxes and hares). Simple trophic models have been of interest to ecologists because they readily capture behaviour that is like that often seen in real ecosystems. Paradoxically, they have been of interest to mathematicians – in part due to their unpredictable behaviour! To illustrate the principles behind a simple trophic model, the example of a lake ecosystem is considered.

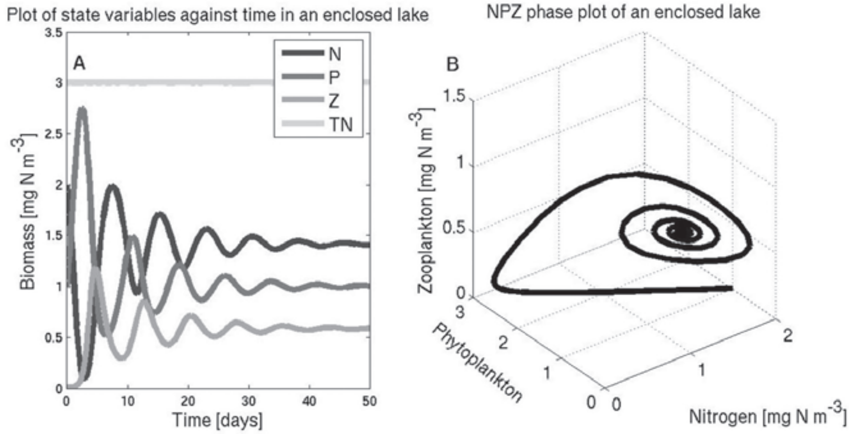


Figure 9.1 The results of the trophic-based ecological model of an enclosed lake described in Section 9.2. a) Nitrogen (N), Phytoplankton (P) and Zooplankton (Z) begin at 1 mg.N.m^{-3}. The P is consumed by Z, releasing N back into the lake, which creates an oscillation in concentrations. After a few weeks, the modelled system stabilises around N ~1.5, P ~ 1 and Z ~0.5 mg.N.m^{-3}. b) The same output in a phase space diagram, showing the attraction of the model to a stable state.

The lake ecosystem is assumed to be made up of three components, N–nitrogen, P–phytoplankton, Z–zooplankton, which will be quantified in mg N m^{-3} (milligrams of nitrogen per cubic metre). Three processes are considered important: phytoplankton growth, zooplankton grazing and zooplankton mortality. In words, the ecosystem model can then be written:

ΔN = – uptake for growth of P + regenerated N from Z mortality
ΔP = + uptake for growth of P – grazing of P by Z.
ΔZ = + grazing of P by Z – mortality of Z

where the Δ symbol represents the change in the value of variable with time. Note that each process appears twice in the equations. For example,

Table 9.1. Definition of terms in trophic modelling.

state variables	a variable whose value changes in time, and describes the state of the system at a given time
parameter	a variable whose value is independent of the value of the state variables
deterministic	a description of a model or equation in which there is no random, or stochastic behaviour
stochastic	a description of a model or equation in which there is a random component

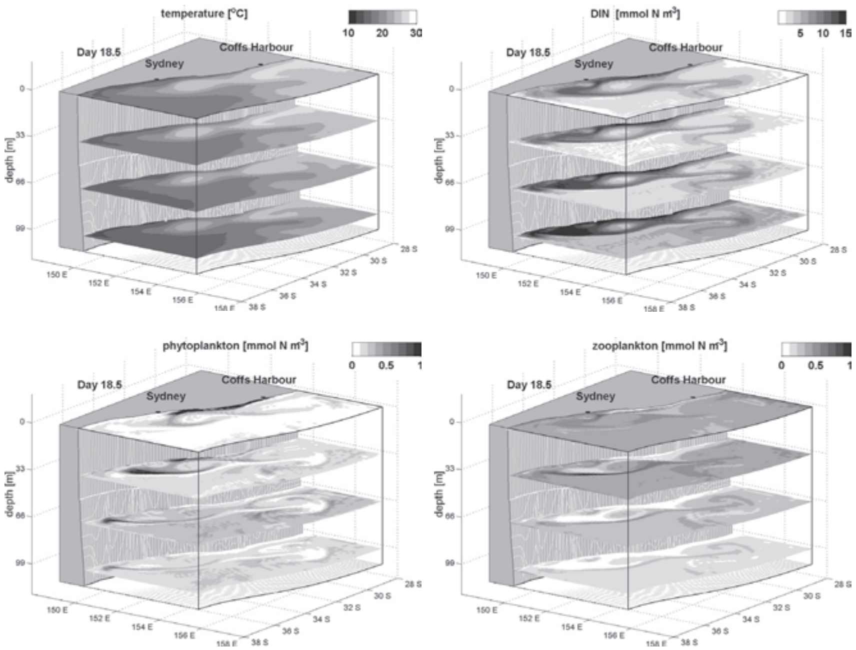


Figure 9.2 The temperature, dissolved inorganic nitrogen, phytoplankton and zooplankton concentration at depth levels of 0, 33, 66, and 99 m on day 18.5 of a simulation of the effect of northerly winds in the waters off south-east Australia. For more details see Baird *et al.* (2006). By day 18.5, cool (top left) and nutrient rich (top right) water has been brought to the surface as a result of the upwelling-favourable winds and become entrained in a warm core eddy. A strong phytoplankton bloom develops along the coast at the surface (bottom left). The bloom ends just north of Sydney, being consumed by a zooplankton bloom as water is advected offshore (bottom right). The zooplankton maximum is just downstream of the edge of the phytoplankton bloom.

grazing of P by Z represents a loss of phytoplankton, but a gain in zooplankton. In this way, mass (in this case N) is exchanged between state variables, but total mass of N within the ecosystem does not change. In other words, the modelled system conserves mass.

In order to create a numerical model, the processes must be represented mathematically. A simple representation is given below:

$$dN/dt = -\mu^{max} NP/(k_{1/2} + N) + mZ$$
$$dP/dt = +\mu^{max} NP/(k_{1/2} + N) - \phi PZ$$
$$d/dt = +\phi PZ - mZ$$

Note how the equations have a similar form to the 'word' equations given above. Apart from the state variables N, P and Z, and t (time), the equations contain the parameters $k_{1/2}$, μ^{max}, ϕ and *m*, which are defined as:

$k_{1/2}$	half saturation of nutrient uptake	1 $mg.N.m^{-3}$
μ^{max}	maximum growth rate of phytoplankton	1 d^{-1}
ϕ	grazing rate coefficient	1 d^{-1} $mg.N^{-1}.m^3$
m	mortality rate of zooplankton	1 d^{-1}

Of course, many other processes, such as phytoplankton mortality or higher order grazing terms, could be considered. The model must now be given initial conditions for each of the state variables. The illustrated simulations will start with initial conditions: $N_0 = 1$ $mg.N.m^{-3}$; $P_0 = 1$ $mg.N.m^{-3}$, $Z_0 = 1$ $mg.N.m^{-3}$. The total amount of mass of the lake is: $TN = N_0 + P_0 + Z_0 = 3$ $mg.N.m^{-3}$ and, given mass conservation in an enclosed lake, will not change. The equations must be solved forward in time, starting with the initial conditions. Figure 9.1 shows the results from the enclosed lake simulation.

Trophic models are commonly coupled to physical models to capture the effects of advection and mixing on biological quantities. The physical models can range in complexity from simple box models with specified transports to advanced hydrodynamic models that use equations of fluid motion to calculate advection (see Figure 9.2 as an example). This rapidly developing field is considered in depth in a recently published textbook (Williams 2006).

9.3 MANAGING PHYTOPLANKTON BLOOMS IN A RESERVOIR BY COUPLED MODELS

The linking or coupling of a model enables the outputs of one model to become the inputs to another. Through linking models, the effects of changes in one part of a system can be simulated in the linked model. Without such links, a model may suffer from the effects at the boundary of the model. One such case is a plankton model, which is influenced by complex processes in the surrounding catchment, but these catchment processes are not often explicitly simulated.

In lakes and reservoirs, excessive phosphorus loads from external sources are a prime cause of eutrophication (Vollenweider 1980). A significant portion of the external phosphorus load originates from the land around these waters, in addition to specific point sources such as municipal wastewater-treatment plants or factories. Lake eutrophication leads to increased biomass of phytoplankton and periphyton (that is, mostly diatom

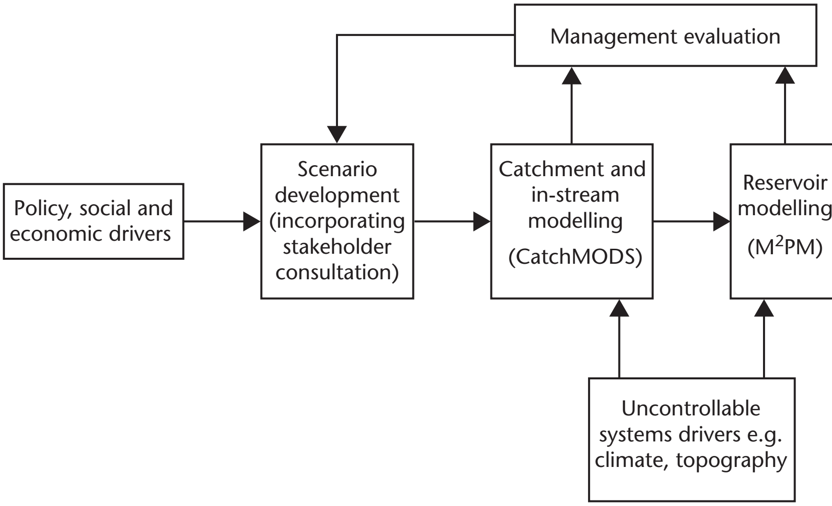


Figure 9.3 Integrated modelling process for formulating and assessing management scenarios. The reservoir modelling contains two models, the nutrient–phytoplankton–zooplankton (NPZ) model, and one for vertical mixing.

films growing on any hard substrate), reduced water clarity, elevated pH and dissolved oxygen depletion in the water column (Smith *et al.* 1999). In particular, bloom-forming cyanobacteria, such as *Microcystis* and *Anabaena*, can change the taste and odour of water, release toxins and clog water filtration systems.

The Ben Chifley reservoir is a medium-sized multi-purpose reservoir located in eastern New South Wales, Australia (Box 9.1). To develop management strategies to reduce the biomass and blooms of problematic phytoplankton (Kobayashi and Church 2003), we conducted a study to assess the environmental and socioeconomic effects of a range of catchment management options, by using a catchment-and-in-stream model, linked with a reservoir model (Figure 9.3), based on measurements of the physics, chemistry and biology in the catchment and reservoir.

The model relies on a comprehensive range of data being collected at appropriate spatial and temporal scales within the catchment and reservoir, and on long time series of data being sourced for these areas where possible. Data for the catchment, included stream water quality and quantity, stream and gully physical dimensions and rates of stream-bank erosion. Data on water quality phytoplankton, zooplankton, climate and hydrology were collected for the reservoir.

BOX 9.1 BEN CHIFLEY CATCHMENT AND BEN CHIFLEY RESERVOIR

Ben Chifley catchment has an area of approximately 985 km^2, with the highest altitude at the eastern and south-eastern margins of the catchment (up to 1330 m above sea level). The Campbells River is the main stream draining the western half of the catchment. Sewells Creek is the main stream draining the eastern side of the catchment. The dominant land use is agriculture, with 65% of the land used as pasture for sheep and cattle grazing and 15% of the catchment is covered with *Pinus radiata* plantations – the remainder is covered by native forest.

Ben Chifley reservoir (149°33′E, 33°34′S) is located at the northern end of the catchment, approximately 20 km south-east of Bathurst. The reservoir was built in 1957. It has an average depth of 5.5 m and volume of 9.2×10^9 L (volume at full supply level is 16×10^9 L). The reservoir is the primary source of potable water for the city of Bathurst and is also used for recreational fishing and water sport activities. Nutrient concentrations of the reservoir indicate that the reservoir is meso-eutrophic (Kobayashi and Church 2003).

A catchment-and-in-stream model – Catchment Scale Management of Distributed Sources (CatchMODS) – estimates pollutant source and transport under current conditions and a variety of changed management scenarios (Figure 9.4). To provide the broad catchment scale perspective required, CatchMODS is based on a series of linked river reaches and associated sub-catchment areas (Newham *et al*. 2004a). In this manner, upstream tributaries provided input for downstream nodes to enable pollutants to be routed through a stream network. Outputs from the model are available for each river reach and are evaluated at the downstream end of the reach. Management recommendations can extend down to these individual river reach and sub-catchment scales. Outputs from CatchMODS include:

- estimates of daily stream flow at each node in the stream network
- a series of summary hydrologic variables (including mean annual stream flow, bank-full stream flow and mean annual base flow)
- total suspended solids (TSS)
- total P
- total N
- the cost (ongoing and fixed) for each management scenario.

Results are reported for each reach in the river network and also the total input to the Ben Chifley Dam. This enabled the outputs of CatchMODS to be linked with the reservoir modelling.

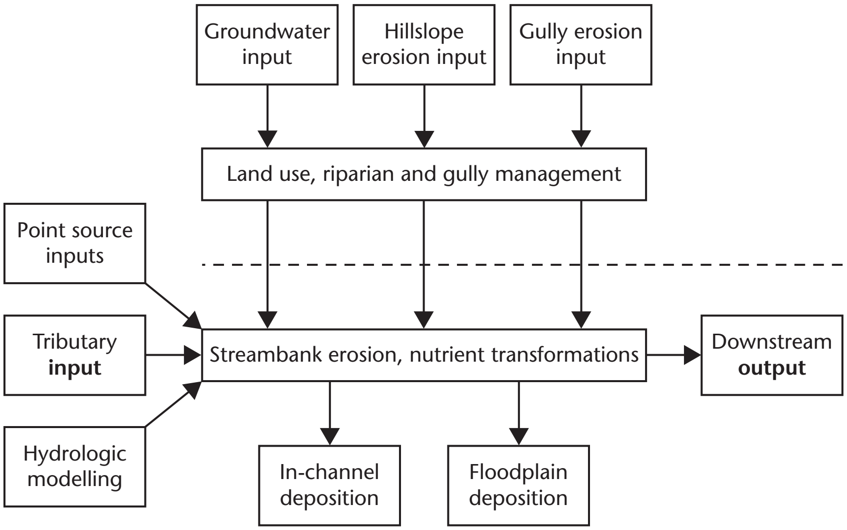


Figure 9.4 Structure of the CatchMODS model. The dashed horizontal line in the centre of the diagram represents the division between catchment and in-stream process modelling. The model links several components – a hydrologic model based on a rainfall-run-off model (Jakeman and Hornberger 1993), a sediment model (modified from Prosser *et al.* 2001), simple total P and total N models and an economic cost component. The downstream output feeds into the reservoir model M^2PM.

The reservoir model consists of a nutrient–phytoplankton–zooplankton model (Berryman 1992) and a vertical-mixing model (Chen *et al.* 1994). Air-water fluxes were calculated from meteorological observations. The coupled Mixing Model and Plankton Model, (M^2PM) was configured with boundary conditions and initial conditions obtained from measurements made in the dam. In this study, the coupled model was integrated with observations that enabled easy comparison with observations and testing of hypotheses specific to Ben Chifley Dam. Outputs from the M^2PM include:

- nutrient concentrations
- plankton biomasses
- dissolved oxygen
- vertical mixing
- water column temperature
- vertical shear in the horizontal currents.

The catchment and in-stream modelling identified priority sites for remediation of diffuse source pollution (Table 9.2). Several sub-catchments

have been identified that have high pollutant source and transport potential relative to the remainder of the catchment. These sub-catchments generally have the greatest stream-flow volumes, and hence potential stream-bank erosion rates, and are also the sites of the highest incidence of gully erosion. They have the potential to contribute large volumes of sediment (and associated pollutants) to the stream network. Because of the proximity of these catchments to the Ben Chifley Reservoir, and minimal floodplain development in these areas, these pollutants also have the highest potential (relative to the remainder of the catchment) to be transported to the reservoir. Simple scenarios constructed for the catchment demonstrate that remediation effort focused on these areas is appreciably more effective than effort that is randomly or uniformly spread throughout the catchment. Remediation efforts could include stock exclusion and establishment of riparian vegetation in gully and riparian zones and more broad land use and pasture management changes. With reducing loads of nutrients from the catchment, the M^2PM indicated a concomitant reduction of phytoplankton biomass (Table 9.2). Note that the use of any plankton model for analysis of a management scenario is only sensible providing one carefully considers the ecological theory upon which the model is based. For the present modelling, the M^2PM was configured with boundary conditions and initial conditions obtained from measurements made in Ben Chifley reservoir.

There is a recognised need to implement integrated approaches to natural resource management (Jakeman and Letcher 2003). An integrated modelling approach to total catchment management is a useful one – improving an understanding of the interactions between terrestrial and aquatic systems. In managing the eutrophication of lakes and reservoirs, linking the catchment-in-stream modelling with the modelling of the plankton population dynamics is of fundamental importance.

9.4 COASTAL LAKE ASSESSMENT AND MANAGEMENT (CLAM) TOOL

Coastal lakes and lagoons often become closed off from the ocean for long periods. As such, they are highly susceptible to catchment inputs and, in New South Wales, Australia, are under increasing pressure from expanding populations. Coastal lake catchments provide a variety of economic, ecological and social values. However, given that the resources are finite, there is increasing conflict over their use and sustainable management. The issues are intricately linked and understanding the impact of making trade-offs and management decisions about coastal lakes and their catchments requires

Table 9.2. Results of CatchMODS and M^2PM model stimulations, for a limited investment scenario (approximately $20 000) showing three hypothetical catchment management scenarios.

	Response variable	Base case	Scenario 1	Scenario 2	Scenario 3
Remediation description	–	Current conditions of land use and riparian and gully management	Conversion of existing grazing areas to forestry use in an upper sub-catchment (Captain King's Creek)	Severe, moderate and minor gully revegetation in a mid sub-catchment (Campbells River 4)	Stream-bank revegetation in lower sub-catchment (Campbells River 2)
Remediation cost	Dollar value	–	$0 (fixed) $20 000 yr^{-1} (ongoing)	$20 000 (fixed) $1670 yr^{-1} (ongoing)	$20 000 (fixed) $2000 yr^{-1} (ongoing)
Catchment response (pollutant delivery to Ben Chifley reservoir)	Sediment load	55 169 × 10^3 kg.yr^{-1}	55 167 × 10^3 kg.yr^{-1} 0% reduction	54 628 × 10^3 kg.yr^{-1} 1.0% reduction	53 999 × 10^3 kg.yr^{-1} 2.1% reduction
	TN load	77 × 10^3 kg.yr^{-1}	0% reduction	1.2% reduction	1.2% reduction
	TP load	1236 × 10^3 kg.yr^{-1}	0% reduction	1.0% reduction	2.1% reduction
Reservoir phytoplankton response (annual mean concentration)	Cyanobacteria (represented by *Microcystis*)	–	no modelled change	1% reduction	1% reduction
	Diatoms (represented by *Aulacoseira*)	–	no modelled change	1% reduction	2% reduction

knowledge of the processes and interactions between all key components of the system. This can be highly complex and requires the integration of information – often minimal – from various disciplines.

The Coastal Lake Assessment and Management (CLAM) approach to developing decision support tools has been formulated to assist decision makers in managing their coastal lake catchments (Newham *et al*. 2004b; Ticehurst *et al.* 2005a, 2005b). CLAM uses a Bayesian decision network (BDN) approach to integrate social, economic and ecological values for the catchment and coastal lake being considered. The approach has been developed to make it, and its outcomes, accessible to managers in a way that any uncertainty associated with data or predictions can be ascertained and understood.

Bayesian networks conceptualise a system through a series of variables joined by causal links (Figure 9.5). Bayesian Decision Networks (BDNs) are Bayesian networks that allow the impacts of individual or cumulative management decisions or scenarios to be explored. Links within the framework represent the relationships between variables. The effects of management scenarios on variables are shown using probability distributions. Probability distributions reflect the likelihood that a particular decision will create a particular response of each variable. Probability distributions have the added benefit of explicitly representing the uncertainty in the relationship between each variable or in the response of each variable to decisions. This allows users to make judgements on the certainty of the model predictions and to assess the risk of making decisions.

The approach also enables the quality of the underlying data to be described and made clear to users. The two ways in which uncertainty is described make it clear to users where information needs to be upgraded or improved, or where acting on the predictions of the models may carry greater risk.

BDNs can efficiently incorporate social, economic and ecological values within the modelling framework because the approach lends itself to the easy incorporation of both qualitative and quantitative data. When observation data or model simulation are not available, expert opinion and local knowledge can be used. The BDN can be readily updated as new information becomes available.

The usefulness of BDNs is increased if they reflect the important processes that operate within each system, but also if the scenarios being assessed reflect the community's and stakeholders' aspirations. By following a specified process, which includes wide consultation with experts and community, it is possible to develop useful models. The process used to

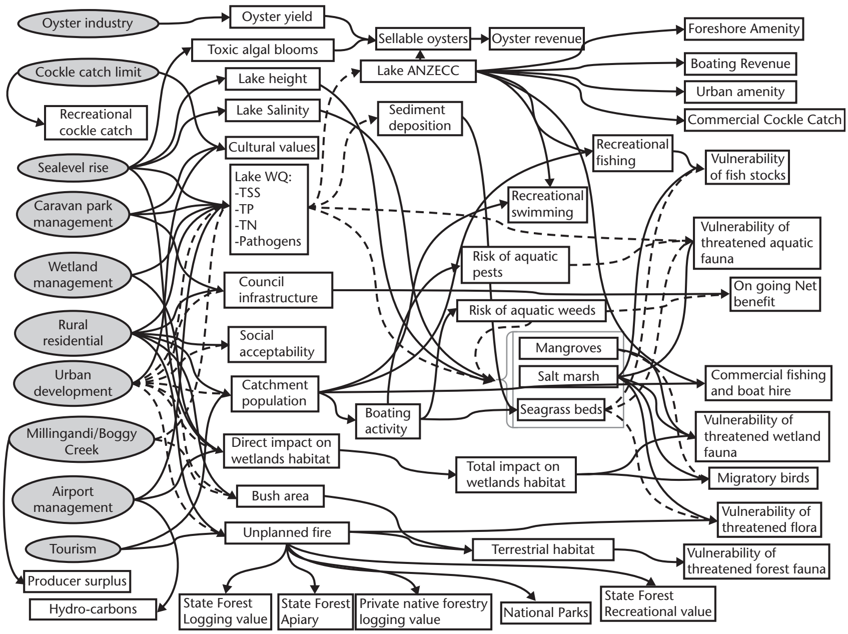


Figure 9.5 Bayesian decision network for the Merimbula Lake CLAM decision support tool. Grey ellipses are decision variables, the dashed and solid lines are equal and are only to assist in the interpretation, 'Lake ANZECC' refers to the water quality guidelines developed by the Australian and New Zealand Environmental Conservation Council, 'Lake WQ' is lake water quality which includes total suspended sediment (TSS), total nitrogen (TN) and total phosphorus (TP).

develop a Coastal Lake Assessment and Management (CLAM) tool for a lake or estuarine system is given in Table 9.3. An initial effort was made to develop a relevant BDN framework following a literature review of appropriate reports and research. Community consultation played an important role throughout the model development process, by providing feedback on the representation of the catchment system and the potential management scenarios to include.

The model input data can be sourced from observed data, model simulation, literature review, general assumptions and expert elicitation. The data are used to create probability distributions for each BDN variable (Stage 5, Table 9.3). The information in each variable of the framework should be calibrated where necessary and possible. Calibration is not necessary for variables with probability distributions determined from on-site observed data. For those variables based on model simulation, models should be calibrated

Table 9.3. Process that should be followed to develop CLAMs.

Stage	Phase
1.	build understanding of constraints, issues and targets for lake and catchment health
2.	develop an initial conceptual framework for BDN and potential future scenarios
3.	review BDN framework with stakeholders
4.	revise initial framework
5.	populate BDN links with data
6.	incorporate the BDN model into a user-friendly software platform
7.	review the interface and populated BDN with stakeholders
8.	revise interface and populated BDN to reflect stakeholder feedback
9.	distribute the sustainability assessment tool to relevant stakeholders with appropriate training in its use

to local values where data are available. Often data about the complex interactions in small coastal catchments are not readily available. In these cases, local experts are used to review variables populated using qualitative data, to ensure that the responses are appropriate for the local conditions.

The CLAM interface is a simple computer software package that has been built in the Integrated Component Modelling System (ICMS). The revised BDN model framework and the probability distributions are coded into ICMS (Stage 6, Table 9.3). The software consists of eight pages, summarised in Table 9.4. Notable features of the software package include:

- photographs, map layers of catchment and lake properties and associated text, which provide the users with information to familiarise themselves with the catchment. These also make the package unique to a particular catchment and help to increase the ownership of the models to local stakeholders and community. This is very important when determining management actions.
- descriptions of the methods and assumptions used to generate the probability distributions for each variable, enabling users to make judgements on the sources of uncertainty in the model input and predictions. This includes a dynamic copy of the BDN framework showing the conceptual structure used within the CLAM DST where users can click on a variable to pop-up a document detailing assumptions made in generating associated conditional probabilities (for example, Figure 9.5). In cases where there are conflicting views related to the impact on a particular variable or the BDN structure,

Table 9.4. Summary of features available in the CLAM software.

Software page	Features available
Welcome	Project background, contacts and licensing agreements
Info	Photograph gallery of the catchment, brief list of facts about the catchment
Maps	Series of catchment properties that can be overlaid, such as land-use protected areas, erosion potential
Approach	Brief description of BDN approach and the BDN framework for the catchment
Inputs	Description of how the probability distributions were attained for each variable, including the assumptions and weaknesses for each
Scenario	Each scenario choice option, plus a map locating various scenarios and a text description of the assumptions used for each scenario
Utility	Change in the dollar value for the economic variables within the model
Output	Resultant probability distribution for each state variable
Report	A summary of the inputs, scenario choices and the output probability distributions, which can be exported and saved

the most supported option is chosen, but both views can be documented in the DST. Therefore the CLAM DST is an example of a 'white-box', rather than a 'black-box' model, as the latter is said to have hidden assumptions.

- display of output probability distributions for each variable. This aspect of the model is important for when liaising with end users; and the function to export and save probability distributions so the user can visually assess the potential impacts of the management scenarios tested.

Given the integrative nature of the model, time series data do not exist to concurrently verify all the BDN variables under current conditions or alternative future scenarios. Instead, the most appropriate method for model verification is for various members of the community and other experts to use the tool and review the input assumptions and the performance of the model predictions and to document their comments.

The CLAM models do not make decisions, but help managers to simplify and depict the complexity of ecological, social and economic factors operating in a systems and its catchment. By using the models, decision makers can understand the potential ramifications of making a decision and can then use the two methods of representing uncertainty to establish the risk of making such decisions. Outcomes may include collecting better data to

increase the certainty associated with the model's prediction, or making a decision with the inclusion of a number of additional management actions to reduce any risk. It is essential to remember that cutting corners during the development of such models, and in the collection of data to populate the models, will reduce their usefulness and effectiveness.

9.5 GENERAL COMMENTS REGARDING HYDRODYNAMIC AND ECOLOGICAL MODELLING

It is important to appreciate that hydrodynamic and ecological modelling are at very different stages in their evolution. The hydrodynamic modelling community has converged on a set of equations that describe fluid motion (the Navier-Stokes equation + Newton's laws of motion). The difference between most hydrodynamic models lies in the mathematical representation of sub-grid scale processes (that is, the summary equations and physical constants used to mathematically describe turbulent closure schemes, friction on boundaries, and so on). The numerical techniques used to solve these equations (finite difference versus finite element etc.) also influence the output of models and the spatial or temporal resolution of model (for example, 1 km or 10 km).

In ecological modelling, there is little consensus on the appropriate model equations to use. Some even doubt the predominant approach of process-based modelling (Falkner and Falkner 2000), although they do not appear to offer a working alternative. Assuming process-based modelling is the way forward, it is easy to see the reasons for the premature state of ecological modelling. In all modelling, a tension exists between an increasingly detailed description of a system whose behaviour may be difficult to interpret, and a simple description that can be more easily understood. In ecological modelling, this tension is amplified by the very complex nature of ecosystems, and emphasis in the biological sciences of cataloguing this complexity. Furthermore, given the fundamentally different make-up of many aquatic ecosystems, the most important processes vary between locations.

What to do? Certainly, results from coupled hydrodynamic-ecological models should be interpreted in the context of the maturity of the two modelling approaches. Furthermore, the ecological modelling community will surely benefit from looking more for simple underlying principles in ecological systems, and making a greater effort to assess different representations of these processes across a broad range of applications. This is certainly a less straightforward task than faced by modellers of fluid phenomena.

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GLOSSARY OF TERMS

alga (plural: algae) chlorophyll-containing plants that lack roots, stems and true leaves; found in aquatic or semi-aquatic habitats; can be microscopic (phytoplankton) or large (seaweed)

anterior in front

aphotic zone portion of a lake beyond the euphotic zone in which respiration exceeds photosynthesis

aquatic invertebrates general term for animals without backbones living in water; includes macroinvertebrates (such as many aquatic insect larvae, snails, mites, etc) and microinvertebrates (zooplankton)

benthos/benthic community of plants and animals living in (or on) bottom

billabong a type of wetland found on river floodplains, formed when river meanders are cut off from the main river channel; also known as oxbow lakes

bioaccumulation the gradual concentration of pollutants as they move through the food chain from one trophic level to the next

bioindicator an organism characteristic of a particular set of environmental conditions, such as high salinity or high nutrients

biomanipulation changing a degraded ecosystem to meet particular management aims, usually by removing fish; this allows zooplankton populations to increase, which in turn reduces algae and allows aquatic plants to re-establish

biomass mass of organisms, often expressed as wet weight, dry weight or carbon weight

biota plants and animals of an environment

bloom a sudden growth of plankton resulting in a distinctive biomass, usually phytoplankton resulting in a red tide

blue-green algae *see* cyanobacteria

carapace shell-like covering of an animal, especially cladocerans, krill, mysids or decapods (prawn or crab), but not in amphipods or isopods

cilia (singular: cilium) many small hair-like structures used for locomotion or feeding by unicellular organisms

ciliates a group of protozoa having cilia in lines

community groups of plants and/or animals sharing the environment; assemblage (*see also* population)

compensation depth the boundary depth between euphotic and aphotic zones where photosynthesis balances respiration, approximately equal to the depth at which the light intensity is one percent of the surface light intensity

concave hollow

copepodite juvenile stage of copepod, after the nauplius stage

corona the circle of cilia surrounding a rotifer's mouth, also called a wheel organ

cosmopolitan worldwide

counting chamber a small recessed chamber used to contain sample of water for microscopic viewing and counting of zooplankton or phytoplankton

cyanobacteria a group of photosynthetic bacteria whose cells lack nuclei, also called blue-green algae (examples are *Microcystis* and *Anabaena*)

cyanotoxins a group of natural toxins produced by cyanobacteria (examples are micocystins and saxitoxins)

detritus dead organic matter derived from plants and animals

diel a behaviour or phenomenon occurring over a full 24 hour period (as distinct from being simply nocturnal or diurnal)

dorsal on back side, opposite to the ventral side where the limbs and mouth/anus typically occur

ephippium (plural: ephippia) a saddle-shaped formation enclosing resting eggs in daphnids (Cladocera); becomes detached from body on death of parent

euphotic zone portion of a lake extending from the surface to a depth where the light intensity is about one percent of that at the surface and photosynthesis exceeds respiration

eurytherms animals that can grow and reproduce well over a wide temperature range (*see also* stenotherms)

eutrophic (of water bodies) rich in nutrients and productive; many lowland lakes and rivers which receive industrial and domestic sewage are often eutrophic (*see also* oligotrophic, mesotrophic)

eutrophication a process of becoming eutrophic; eutrophication caused by human activities is often called cultural eutrophication; usually resulting in a community dominated by phytoplankton

exuviae outer shell or skin of invertebrates discarded during growth by moulting, such as cladoceran carapaces

flagella (singular: flagellum) fine, long whip-like appendages used for movement

flexion stage of larval fish development when the notochord (precursor to the backbone) bends slightly upwards (dorsally) to form the tail fin

formaldehyde a solution of formaldehyde used for preserving biological samples – long-term exposure is carcinogenic

freshwater water with less than 5% seawater or less than 3 g L^{-1} of dissolved salt

fusiform spindle-shaped; broad at the middle and narrowing towards the ends

gelatinous jelly-like

genus (plural: genera) a major subdivision of a Family of organisms, comprising one or more species

HAB harmful algal bloom

incudate a type of rotifer mastax (or trophi) with a seizing, pincer-like shape, characteristic of carnivorous animals

larva (plural: larvae) young of invertebrates, usually different in form to adult

limnetic zone *see* pelagic zone

limnology the study of inland waters and their ecology

littoral zone shore of river or lake inundated for some or all of the time; the shallow-water region extending from the shore to a depth where light is sufficient for rooted aquatic plants to growth

lorica a firm shell covering the body of a rotifer or protozoan and some algae

macrophyte large plant of any type

malleoramate a type of rotifer mastax (or trophi) with many teeth attached to the base structure

mastax mouthparts of rotifers (*see also* malleoramate, uncinate)

metazoan a multicellular animal

mesotrophic (of water bodies) middle level of nutrients and moderately productive (*see also* eutrophic, oligotrophic)

micron one micrometre (1×10^{-6} m) or a thousandth of a millimeter. Also shown as µm

moulting shedding outer skin during invertebrate growth

multicellular consisting of many cells (*see also* unicellular)

nauplius earliest stage of crustacean larva (such as a copepod) after hatching from an egg

nekton small animals with a good swimming ability, such as krill or jellyfish

neuston plankton that occur at or just underneath the surface

oligotrophic (of water bodies) poor in nutrients and least productive; many undisturbed highland lakes are oligotrophic (*see also* eutrophic, mesotrophic)

omnivorous eating both plants and animals

parthenogenesis reproduction in which eggs do not require fertilisation by male sperm

pelagic zone the open-water region of lakes and other water bodies, also termed limnetic zone

pH values values related to the concentration of hydrogen ions in water; the higher the pH value, the fewer hydrogen ions; water is called acidic if the pH values are 1–7, neutral (pH value near 7) or alkaline if the pH values are 7–14

phylum (plural: phyla) a major subdivision of a Kingdom, comprising one or more Classes of organisms. Approximately 32 phyla of animals on the planet today

piscivorous fish-eating

planktivorous plankton-eating

plankton drifting organisms including phytoplankton (floating algae) and zooplankton (floating animals)

population group of individuals of a single species (*see also* community)

posterior rear (hind) side

primary production production of organic matter from inorganic materials, usually by photosynthesis

pseudopodia (singular: pseudopodium) temporary foot-like protrusions of a protozoan

red (or brown) tide a bloom of phytoplankton with reddish (or brown) pigments

resting eggs fertilised eggs with thick shell in rotifers and cladocerans

rostrum pointed part of the carapace, extending between the eyes

Sedgwick Rafter counting cell *see* counting chamber

sessile attached to the surface of an object (e.g. rock or boat)

setae small bristles or spines

solitary individual (*see* colonial)

species basic classification of a group of organisms having some characteristics in common (sometimes abbreviated as sp. or spp. when only the genus is known)

species richness number of species in a sample or habitat

stalk a stem-like structure connecting the body of a protozoan to other animals or substrates

statocyst a balance organ to sense gravity

stenotherms (of organisms) tolerating only a narrow temperature range; can be warm stenotherms (requiring warm water) or cold stenotherms (requiring cold water) (*see also* eurytherms)

suspended solids the very small particles of inorganic and organic material in a water body

symbiotic living together in more or less close association or even union

taxonomy the science of classifying organisms

telson the last or terminal segment of a crustacean

test a rigid shell covering the body of a protozoan or invertebrate

thoracic middle segment(s) of invertebrate body to which limbs are attached

uncinate a type of rotifer mastax (or trophi) with three to five pairs of tearing-type teeth attached to the base structure, found only in the Family Collothecidae

unicellular single-celled (*see also* multicellular)

vector an organism that transmits germs or other agents of disease

ventral on abdominal (front) side

zooplanktivorous zooplankton-eating

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